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MICROBIOLOGICAL STUDIES OF APPALACHIAN PODSOL SOILS

III. SYNCHRONOUS CHANGES OF BACTERIAL NUMBERS IN TWO FIELD SOILS[1]

BY P. H. H. GRAY[2]

Abstract

Determinations of the numbers of bacteria (and actinomyces) in two cultivated podsol soils, sampled on the same days at intervals of one month during the growing season of 1937, showed that numbers developing in plates of two agar mediums fluctuated extensively and in the same manner in both soils. The fluctuations were of greater amplitude in plots treated several months previously with limestone, with sodium carbonate, and with both amendments together, than in the control plots. Numbers in the late spring (June 14) were from two to four times the numbers found in the summer (August 11). In the spring the less selective medium (soil-extract–dextrose agar) gave higher numbers than the more selective medium (Thornton's mannitol-asparagine), but in the summer the numbers were the same in the two mediums.

Introduction

The main purpose of estimating the density of the soil microflora has been to find some relation between numbers of organisms and the factors affecting crop yield. This method of approach has not hitherto proved to be sound, since the plating method restricts the number or kinds of micro-organisms entering into the estimate, and since seasonal changes in numbers have proved to be of such amplitude that numbers found at one time cannot be accepted as representing the actual state of the flora at all other times during the growth of the crop.

The plating method does, however, give evidence of the effects of some treatments, such as liming, that are expected to affect crop yields. Liming appears to encourage the activity of the nitrifying bacteria, but there appears to be no evidence that lime added to field soil stimulates the decomposition of organic compounds of nitrogen and the production of ammonia therefrom, thus furnishing the nitrifying bacteria with increased supplies of oxidizable nitrogen, although the numbers of aerobic heterotrophic bacteria usually associated with such decompositions may be increased by liming (1, 6). Other soil amendments, such as calcium oxide and sodium carbonate, which release organic matter in acid soils (7), have also been shown to stimulate nitrification as well as the multiplication of the heterotrophic bacteria in field soils (1).

[1] *Original manuscript received January 29, 1938.*

Contribution from the Faculty of Agriculture of McGill University, Macdonald College, Que., Canada. Macdonald College Journal Series No. 93. This paper is the third in a series dealing with part of the work of the Soil Fertility Committee of Macdonald College.

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Jensen (5) has recently shown that the numbers of bacteria, actinomycetes and fungi in 50 cultivated soils of New South Wales, Australia, were separately correlated with the organic matter. The relationship was also shown to exist in the separate horizons of heavily podsolized soils of Quebec (2, 3). Such relationships as have been reported, however, have been based on that between the micro-organisms and the total organic matter, as determined by ignition; a better understanding of the relation between the heterotrophic soil flora and the organic matter may be attained only after analysis of the material in the soil solution. It is also necessary that such a relation be established after knowledge has been attained as to the magnitude of the fluctuations that take place seasonally among the organisms.

Seasonal fluctuations of microbial activity in Appalachian podsol soils have been reported (2), as occurring synchronously in soils in areas as much as 25 miles apart. In that work, the samples of soil were collected at any one place 14 days after collecting samples at the place previously visited. The results, though clearly pointing to the existence of fluctuations of the same order and in the same direction, in soils so widely separated, remain open to criticism in that there was no evidence that fluctuations of some extent did not occur during the six-week intervals between the times of sampling at any one place.

In attempting to show the existence of an association between the density of the microflora and available organic matter, two lines of approach have been chosen; first, to determine the density of micro-organisms in soils receiving such amendments as are known (6) to release organic matter; and, secondly, to add easily decomposable compounds of carbon and nitrogen to soil and then to determine the subsequent biological activity. The studies reported below were concerned with the former of these lines of approach.

Experimental

The present work was undertaken to ascertain whether fluctuations occurring at one place would be the same, either in degree or direction, as those occurring at another place where samples were taken on the same day. In order to obtain samples of a uniform nature, advantage was taken of plots laid out in October 1935, at two farms near Sawyerville in the Eastern Townships of the province of Quebec, for the purpose of studying the effects of limestone and sodium carbonate, alone or in combination, upon the soil and crop yields. The soils were situated a few miles apart, and were of similar origin; they differed in that one had only recently been brought into cultivation.

The plots at each place were 16 in number, each 1/200 acre in area, and were arranged in 4 rows, one plot in each row being kept without treatment. The plots were sown with oats, seeded to clover and timothy grass, in the spring of 1936. Samples for the work reported here were taken first in 1937 on the dates shown in the tables. Five cores of soil, 6 in. deep, were taken from each of the four plots and thoroughly mixed; from the composite samples of 20

cores, a sample of about 4–5 lb. was packed in a can for determinations in the laboratory; the sample usually reached the laboratory within 48 hr. of sampling. In the laboratory the whole composite sample was passed through a 3-mm. sieve, and determinations were made for moisture in fresh soil, pH, and numbers of bacteria and actinomyces developing in two agar mediums.

Since the values for moisture in fresh samples were used merely to assist in estimating results on a basis of dry soil, these values are not quoted. Also, since the present paper does not aim to consider the effects of treatment on biological activity, the pH values of the samples are not reported. It may be mentioned, however, that the moisture content of the soil, *T*, recently brought under cultivation, was consistently higher than that of the other soil, *R*, and that the treatments with ground limestone, or sodium carbonate, or both, raised the pH values from about 5.0 to 6.5. Such fluctuations of the values of these two factors as were found could not be associated with the fluctuations in microbial activity.

The mediums used were Thornton's mannitol-asparagine medium and soil-extract–dextrose agar. The former medium was selected for its known value in detecting significant fluctuations in numbers of bacteria in soils of varied types; soil-extract–dextrose agar was selected for the reason that, being less selective, it might prove useful in determining the density of larger groups of bacteria especially associated with the decomposition of organic compounds.

TABLE I

BACTERIA AND ACTINOMYCES, MILLIONS PER GM., THORNTON'S MEDIUM

Plots	May 13	June 4	July 13	Aug. 11	Sept. 16
			Soil R		
Control	6.02	10.15	5.27	5.84	7.37
Limestone	14.40	16.94	9.17	6.55	13.14
Sodium carbonate	8.76†	19.85†	11.76	4.19	22.59
Both amendments	14.63	27.21	15.57	6.30	9.42
			Soil T		
Control	10.85	12.40	8.89†	8.77	21.45
Limestone	31.39	48.25	30.31	14.79	16.96
Sodium carbonate	26.99	40.63	20.65	16.05†	8.81
Both amendments	36.64	40.84	21.92†	13.13	17.73†

† χ^2 *excessive;* $P < 0.02$.

The soil extract was prepared by autoclaving 1 kg. of soil with 1000 ml. of distilled water, filtering hot through filter paper, and making the filtrate up to 800 ml. To this extract, K_2HPO_4, 0.02%, and agar 1.5% were added. Before sterilizing, dextrose, 0.5%, was added; the reaction was not altered. Each medium was usually sterilized in measured 50 ml. quantities in Erlenmeyer flasks of 125 ml. capacity, and melted just before use.

The numbers of bacteria and actinomyces, estimated by counting colonies developing in five plates after ten days at 25° C., are given in Tables I and II.

TABLE II

BACTERIA AND ACTINOMYCES, MILLIONS PER GM. SOIL EXTRACT MEDIUM

Plots	May 13	June 14	July 13	Aug. 11	Sept. 16
			Soil R		
Control	26.8	15.6	5.7	6.5	15.2
Limestone	(67.0)*	28.6	9.1	9.6	19.9††
Sodium carbonate	38.4	48.8	11.8	5.8	30.6††
Both amendments	(82.0)*	Lost	19.5	6.7	19.0
			Soil T		
Control	21.0	26.4	18.1	10.5	44.9
Limestone	48.3	56.1	33.4	12.9	28.0††
Sodium carbonate	(48.3)†	59.3	16.4	13.4	22.5
Both amendments	(60.3)†	50.4	24.0	11.8	28.8††

** More than 500 colonies.* *† From single plates. These values were omitted in calculating the average numbers in the treated plots, as shown in Fig. 2.*

†† χ^2 excessive; $P < 0.02$.

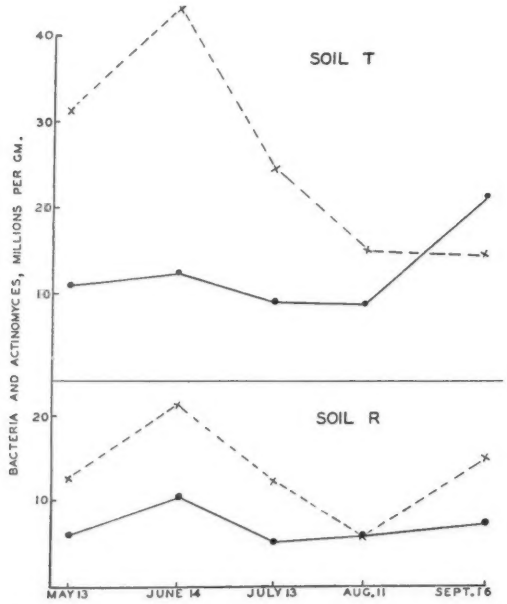

FIG. 1. *Changes of bacterial numbers in 1937, in Soils R and T; Thornton's medium. Solid line, control plots; broken line, treated plots.*

The values estimated from the number of colonies found in only one plate of a series (the remainder were spoiled by fungi or spreading colonies) and those estimated from the mean numbers of colonies in plates, the χ^2 of which was excessive, are noted in the tables.

The results for numbers of bacteria and actinomyces are shown also by means of graphs in Figs. 1–3.

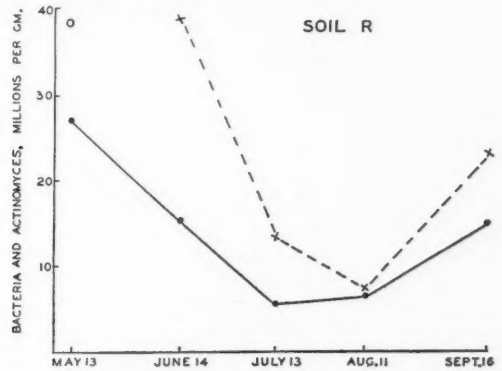

FIG. 2. *Changes of bacterial numbers in 1937, in Soil R; soil-extract agar. Circle, sodium carbonate plots only. Solid line, control plots; broken line, treated plots.*

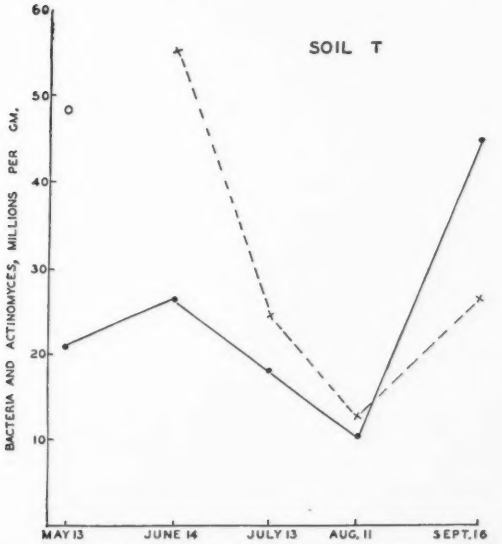

FIG. 3. *Changes of bacterial numbers in 1937, in Soil T; soil-extract agar. Circle, limestone plots only. Solid line, control plots; broken line, treated plots.*

Conclusions

The two soils differed in biological activity, in that the mean level of numbers of bacteria and actinomyces was higher in soil T, which had been brought into cultivation only a few months preceding the year of sampling. The mean levels of numbers are shown in Table III.

TABLE III
BACTERIA AND ACTINOMYCES, MILLIONS PER GM.

	Thornton's medium	Soil extract medium
Soil T	10.2	19.0
Soil R	6.8	13.7

The values are the average of the numbers of organisms in Samplings I to IV from the plot that had not been treated; the values from Sampling V are omitted, since there was an unavoidable interval of five days between collecting and plating.

There appears to have been a common factor affecting the numbers of micro-organisms throughout the season at the two places. Considering the more selective medium first, there was a rise in numbers in the middle of June, followed by a fall, which was maintained for two months. There appears to have been a tendency for numbers to increase in September, the average of the numbers in Soil R in that month being more than twice those in August. The fluctuations were greater in the treated plots, and of relatively greater amplitude in Soil R than in Soil T, though numbers in the latter soil were consistently the higher.

Unfortunately, some of the values obtained by plating with the less selective soil-extract medium cannot be accepted as reliable. In spite of these, however, two points of interest seem to call for notice: first, the higher numbers developing at first in this medium as compared with the more selective medium were not maintained throughout the season, notably in August, when they were about the same in the two mediums; and, secondly, in September the numbers were twice or three times as great as in August.

The results obtained by the use of the less selective medium are of further interest in that the numbers in the treated plots in the August sample were at the same level as those in the control plots.

The ratios between the numbers in the June samplings and the August samplings are found to be 4 : 1 in the soil extract agar, and 3 : 1 in the mannitol-asparagine medium. It would appear from this that groups of bacteria that were active in the spring in both soils were removed by factors common to both soils. Some of the bacteria were evidently those stimulated by the limestone and sodium carbonate, the effects of which were operative mainly in the spring.

It is possible to compare these results with those reported previously for one soil situated in the same locality (Soil S in Reference (2)). The changes in 1931 were unlike those of 1937, only in that in the earlier year higher numbers were found in May than in June; in 1932, the higher numbers occurred

in August; the fluctuations in 1933 resembled those of 1937 in direction but not in degree.

In spite, therefore, of the agreement found in the nature of bacterial activity in two widely separated soils, it does not seem possible to accept the changes found to occur in any one year as part of a normal recurring cycle, which can be ascribed to external climatic influences in these soils.

Acknowledgment

Thanks are due to Mr. R. J. D. Martin, who was employed as technician during this work.

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QUALITATIVE STUDIES OF SOIL MICRO-ORGANISMS

I. GENERAL INTRODUCTION[1]

By A. G. Lochhead[2] and C. B. Taylor[3]

Abstract

Soil microbiological research has been directed for the most part towards a study of processes in which micro-organisms are known to participate rather than towards an objective study of soil micro-organisms themselves. While organisms concerned with known processes have been given much study, relatively little attention has been paid to groups of bacteria whose functions are as yet unknown or but little understood, but which are believed to comprise a very large proportion of the micro-population of arable soils. A review is made of investigations based on the biological, as contrasted with the biochemical (or functional) approach to soil microbiology. Qualitative studies of the general soil microflora are regarded as essential to a better understanding of microbiological activity in soil and its relation to practical problems of crop growth, soil borne plant diseases, and general soil fertility.

Approach to Soil Microbiology

Soil microbiological research in the main has been directed towards studying microbiological processes rather than the micro-organisms themselves. The rise of bacteriology in the latter half of the nineteenth century led to an immediate and phenomenal application to medicine which gave hopes of an equally effective application of the new science to problems of soil fertility. Coincident with the discoveries, in the closing decades of the century, of the role of bacteria in human and animal disease, equally brilliant if less spectacular discoveries were made of the part played by micro-organisms in many soil processes.

Right up to the present the study of processes, and incidentally that of the specialized groups of organisms concerned in these processes, has occupied by far the greater part of the attention of most soil microbiologists. Valuable data have been gathered on the numerous biological processes known to occur in soils, such as ammonification, nitrification, nitrogen-fixation, processes concerned with the transformation of sulphur and other elements, the decomposition of plant residues and miscellaneous organic compounds. Detailed investigations have been made of bacteria and other micro-organisms known to take part in such processes. However, such organisms have been studied, not so much from an interest in them as organisms, but because of their known, and presumably important functions.

The immediate application of bacteriology to medicine, and a similar concentration, in the case of soil microbiology, on functions, has if anything delayed progress in the objective study of bacteria. Even today, when microbiology is so widespread in its application, many of the fundamental questions of the morphology and physiology of bacteria remain unanswered or at least in active dispute.

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Contribution No. 47 (Journal Series) from the Division of Bacteriology, Dominion Experimental Farms, Ottawa.
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[3] *Graduate Assistant.*

In soil, bacteria are indigenous to the medium in a sense not true of bacteria in milk, in foodstuffs or in blood or living tissues. In arable soil we have a centuries-old equilibrium, though admittedly an ever-changing one, not analogous to conditions prevailing, *e.g.*, in a given sample of milk or in an infected animal. In the latter instances, too, we can better recognize cause and effect; we have to deal with fewer antagonisms and associations of different groups of organisms; and we have a better conception of the functions of the organisms in the medium. In the soil we can perceive many biological processes. Some of these we can evaluate fairly well; others, such as non-symbiotic nitrogen-fixation, we are unable to evaluate though the organisms able to exercise the function under artificial conditions have been given much study. There is reason to believe, however, that the organisms in soil which take part in recognized soil functions are greatly outnumbered by those whose functions are yet unknown.

Greater cultural difficulties doubtless stand in the way of a biological, as contrasted with a biochemical (or functional) approach to soil microbiology. We require a non-selective rather than a selective medium to permit of the isolation and study of the greatest numbers of types occurring in soil. Much more success has been achieved in the development of highly selective culture media than in the development of non-selective media, and of the latter type the most we can say is that they are less selective than the others.

Our least selective media are used for the most part for "total counts" of soil organisms. The enumeration of plate colonies constitutes the only consideration usually given to many types of micro-organisms characteristic of soils and forming a large part of their total population. The importance of many types in soil is likely to be gauged by the size of their respective colonies, very many of which are small or of pin-point size. This impression may be strengthened by casual microscopic and physiological tests which indicate, in a large number of cases, small, rod-shaped organisms, relatively inactive as judged by our standard artificial testing procedures. At the present state of our knowledge, however, micro-organisms that do not appear to fit into our more important physiological groups as we recognize them today, cannot be dismissed as unimportant. More study of an objective nature is required before we can reliably assess such groups. Their undoubted abundance in most soils renders essential a more thorough knowledge of them than we possess at present; such knowledge would help to form the basis of a more complete understanding of soil micro-organisms in general.

The General Bacterial Microflora of Soil*

Our present knowledge of the general microflora of the soil, as distinct from types of micro-organisms concerned with known biochemical processes, is due to a comparatively small number of investigators, and in a large measure

* *This paper is concerned primarily with bacteria, the most abundant soil organisms and the group studied relatively least from the purely qualitative point of view. It is recognized that actinomyces, fungi, algae and protozoa comprise important divisions of the micro-population of soil.*

to the work of Conn, who extended and developed ideas underlying the earlier work of Chester. Previous investigators, to be sure, had reported studies of bacteria isolated from soil. As early as 1881, R. Koch (26) made the observation, which has been repeatedly confirmed since, that rod forms are greatly in excess of cocci. As was but natural from the prominence of their colonies on beef gelatine or agar plates largely employed in earlier work, spore-formers received particular attention. Houston (21) describes various types of this group, devoting less attention to non-spore-forming organisms. He and other contemporaries of Chester, such as Gottheil (19) and Neide (31) who studied spore-formers, were apparently more interested in definite groups than in the whole microflora of soils.

In 1900 Chester (1) first emphasized the importance of a knowledge of the types of organisms predominating in soil. Introducing a study on bacterial classification he enunciated a principle which has received all too scant attention.

"Agricultural bacteriology is destined to have a very important bearing, but as yet is without any foundation. The animal pathologist deals with a comparatively few forms which he can readily identify. The agricultural bacteriologist, on the other hand, can scarcely take up a piece of work before he meets with scores of bacterial forms of which he knows nothing, and which he is unable to identify. Hence the first desideratum before he can advance in this important field is to possess some system of bacterial classification, however crude and imperfect. These studies in bacterial classification have been preliminary to the investigation of the bacterial flora of cultivated soils. Inasmuch as soil bacteria are the active agents for the digestion and elaboration of plant food in soil it is important to know something about them, not only collectively but individually. It is necessary to know what species of bacteria are commonly present in all soils and the part each plays in plant food elaboration."

In the same year Chester (2) described miscellaneous types of bacteria isolated from soil, and in 1903 (3) published what is probably the first study of the predominating bacteria of soil. From gelatine plates of 1/100,000 dilution of soil, giving but small numbers of colonies, the predominating types of organisms were studied in detail. The three main types in order of abundance were named, following Migula's system of classification, *Streptothrix soli*, *Bacterium flocosum* (a non-motile spore-former) and *Bacillus Delavariensis* (a motile non-spore-former). This and related studies by Chester (4), such as a special investigation of spore-forming bacteria in soil, represent the most important work up to that time on the qualitative nature of the soil microflora. It was pointed out by the same author (5) that in order to form a true estimate of what is taking place in soil through the agency of bacteria we should understand the function of the different types. He stressed the importance of isolating all types which predominate in soil and of a quanti-qualitative soil microbiological analysis.

Chester was breaking new ground while bacteriological methods were still far from perfect. The principles involved in his work are perhaps more

important than the findings obtained, and deserve more consideration than has been accorded up to the present.

In 1903 Hiltner and Störmer (20) made a study of types of bacteria in soil, on lines of general groups rather than of definite species. Three main groups were recognized, liquefiers, non-liquefiers and Streptothrix. The numerical importance of spore-formers in soil was put in doubt by studies which showed that they comprised but a small percentage of colonies on plates whereas non-liquefying, non-spore-forming organisms formed by far the largest group. Whereas Chester (5) inclined to the belief that the prevalence of kinds of bacteria in a soil was largely a fortuitous matter, with relatively few species predominating, Hiltner and Störmer found the relative numbers of the broad groups to be fairly constant in normal soils, suggestive of a certain state of equilibrium.

In a series of studies first appearing in 1917, Conn (7, 8, 9, 10, 12) added much to our qualitative knowledge of soil bacteria through extensive work on the general soil flora as contrasted with the more intensive work on special groups of organisms that were considered important on account of their physiological activities and commanded most attention from contemporary soil biologists. Conn's provisional classification recognized five main groups in soil as determined by studies of colonies on gelatine and agar plates:—(i) spore-formers, (ii) rapidly liquefying, non-spore-forming short rods, (iii) slowly liquefying or non-liquefying, non-spore-forming short rods, (iv) micrococci and (v) Actinomyces. Of these, Groups (ii), (iii) and (v) were the most numerous, the slowly liquefying or non-liquefying short rods being the most abundant and doubtless comprising the same broad group recognized by Hiltner and Störmer. Martin (30) likewise found non-spore-formers to comprise the majority of organisms in normal soil with Actinomyces next in abundance, and spore-formers occurring in smaller numbers. In a study of Texas soils, however, Williams (38) reported having found spore-forming bacteria as the dominant types from an examination of colonies isolated. However, as no attempt was made to determine the relative abundance of different forms occurring on plates it is not possible to assess the relative incidence of the various types.

In studying the predominant types occurring in frozen soil Lochhead (29) showed the largest group to consist of slowly liquefying or non-liquefying, non-chromogenic short rods, which group represented in even more pronounced degree the majority of bacteria capable of growth at low temperature (3° C.). Actinomyces, though unable to grow at low temperature, comprised the second largest group. Non-spore-forming, liquefying short rods, spore-formers and micrococci formed numerically much less important groups. The relative abundance of the different groups in frozen soil corresponded closely with the findings of Conn and led to the belief that, as far as the main types are concerned, the winter flora of soils differs little from the summer flora.

The rapidly liquefying short rods, Group (ii) of Conn's classification, were apparently closely related to *Pseudomonas fluorescens*. Though forming a

relatively small portion of the total microflora, they were found by Conn to be specially abundant in freshly manured soil, a finding confirmed by the work of Lewis (28), and with their distinctly proteolytic properties were suggested as being important soil ammonifiers.

The great majority of the non-spore-forming organisms, made up largely of the group of slowly liquefying or non-liquefying short rods, were less active physiologically and grew less abundantly on the media used. Organisms of this group were referred to at first as "slow growers" by Conn, and later as "punctiform colony forming bacteria" on account of the restricted size of colonies on tap water gelatine. While several sub-divisions of this group were made (14), special attention was given to two types which embraced the great majority of the forms studied:

(I) Small, short rods, motile or non-motile, with no tendency to produce irregular forms. With this sub-group many variations in staining properties and physiology may be observed. This suggests either the existence of many species within the sub-group or unstable physiological characters.

(II) Pleomorphic forms, appearing as short rods in young culture but changing into cocci within a few days. While variability in staining and physiology occurs, it is less pronounced than in the previous sub-group.

In addition to the above, other much less abundant sub-groups (III and IV) were noted, less definitely classifiable, but showing a tendency to produce filaments, branched or unbranched. These forms are doubtless related to soil organisms showing branching and described as members of the genera *Corynebacterium*, *Mycobacterium* and *Proactinomyces* by Jensen (22). It is probable that such organisms with tendency to produce branched forms may comprise relatively large proportions of the micro-population in some soils. Thus in Australian soils Jensen (23) found corynebacteria to comprise from 8 to 65% of the colonies appearing on dextrose agar plates. *Mycobacteria*, however, were found by Jensen (24) to occur much less frequently, though Krassilnikow (27) states that they are widely distributed in certain Russian soils, especially those rich in humic substances.

Organisms of the sub-group (II) above, comprising the cocci-forming rods, gave evidence of a much closer inter-relationship than those of (I) and were regarded as consisting almost wholly of one species, to which the name of *Bacterium globiforme* was given. To this organism, one of the predominating types in the soils studied, special attention has been given by Conn (13, 14) and Conn and Darrow (16, 17), particularly in view of its apparently greater abundance in many productive soils than in certain less productive soils investigated.

Even in soils that may be classed as abnormal the main groups recognized by Conn appear to be present, though under extreme conditions such as are represented by arid or desert soils the proportions may be considerably altered. In a series of investigations summarized by Snow (33) studies were made of the bacterial flora of wind-blown soils from six localities. In but one of the soils studied was the average total count in excess of 100,000 per gram.

Examination of the types isolated suggested that cocci, liquefying short rods, and in most cases spore-forming rods, formed relatively larger proportions of the cultures from such soils than of those from more arable soils.

Coincident with progress in soil flora studies from the cultural side has been a development of methods for the direct microscopic study of soil organisms. The first method, proposed by Conn (11), was essentially an adaptation of the Breed smear method for milk examination, and consisted in the staining of a suspension of soil after fixing and drying on a slide. This procedure, with some modification, was later used by Winogradsky (40, 41) in connection with his "direct method" of soil study. A very important development was made by Rossi and Riccardo (32) who first advocated the use of the direct contact slide method and provided a new means of studying not only the forms, but also colonies of micro-organisms, as they occur in soil, and other points of interest not brought out by the stained suspension method. A very important modification of this, the most direct method, was made by Cholodny (6), while further adaptations were suggested by Conn (15) and others for the examination of soil *in situ* or in the laboratory, with any desired modification or treatment.

By the aid of the microscopic method, Winogradsky (39, 41) was able to study the main morphologic types in soil and particularly their reaction to changes in environment, such as are caused by the addition of nutrient materials. He classified soil organisms in two main categories. One consists of the *autochthonous* (*i.e.*, indigenous) flora, characteristic of soils poorly supplied with fermentable substances. Organisms of this group are for the most part oval forms or cocci, comparatively inactive and believed to take part in the slow combustion of the humic constituents of soil. Another category was recognized which he calls the *zymogenic* organisms. These are scarce in normal soils but become very active upon addition of any readily fermentable substance. In this group are included the spore-formers, which as Joffe and Conn (25) had shown, are apparently inactive under ordinary field conditions but may multiply upon addition of fresh available organic matter, particularly when abundant moisture is present, and engage in decomposition processes.

Apparently Conn's group of non-spore-forming bacteria corresponds essentially to Winogradsky's autochthonous group, representing the indigenous soil organisms as contrasted with other types which come into prominence mainly under special conditions. The agreement at first was not so evident. Winogradsky assumed that the autochthonous group was largely incapable of being cultivated on ordinary media from the fact that, whereas cocci forms predominated in the microscopic examination of soil, relatively few cocci developed in cultures. However, Thatcher and Conn (35) found that in some soils as many as 40% of the organisms growing on plates may consist of coccus-forming rods. This work was followed by the studies of Conn, and Conn and Darrow, referred to above, and by the recognition of the *Bacterium globiforme* group as an important part of the autochthonous soil flora.

Further application of the microscopic method has been made by various workers in studying the relative abundance of different groups in soil, particularly as affected by soil treatment. Thus the work of Demeter and Mossel (18) and of Vandecaveye and Villanueva (37), though carried out by different modifications, showed that useful application could be made in indicating qualitative changes on a broad basis with approximative quantitative values. The method, however, is inadequate for studies of the role of the organisms in soil. The soil slide method provides us, to be sure, with an additional and valuable means for soil flora investigation. It possesses certain advantages and also the limitations of microscopic methods. From a qualitative point of view it may be used to advantage in noting the prevalence of different morphologic types and their reaction to environmental changes. It is therefore a useful supplement to cultural studies, but the latter, however, are necessary for an adequate study of the unknown organisms in soil and their possible functions.

Of the autochthonous microflora it would appear that *Bacterium globiforme* (or the *Bact. globiforme* group) comprises a significant part, though relatively little attention has been accorded it. Conn (13, 14, 16, 17) studied the physiological properties of the organism and furthermore noted certain relationships between its incidence and soil productivity. Of interest was its occurrence in certain fertile soils and its absence from certain less productive soils, suggesting that the inability of the latter to support growth of *Bact. globiforme* was associated with their relatively low crop-producing ability. The work of Conn and Darrow (16) suggested further that the growth of the organism in soil was dependent upon the presence of readily available nitrogen, which is lacking in the poor soils. Further work by the same authors (17) led to the conclusion that the organism retains, in the soil, nitrogen that has been converted by other organisms into a soluble form and which otherwise would be removed by drainage or utilized by plants. Depending upon conditions, therefore, the organism may be beneficial or harmful, with the beneficial function predominating.

In comparing the incidence of *Bact. globiforme* in soils differing in fertility, Taylor and Lochhead (34) found, by quanti-qualitative methods, no indication of relationship between the abundance of the organism and the productivity of the soils in question. There was more indication of an influence of the crop on the bacterium, though in all cases it formed a significant part of the total microflora. The findings obtained, when compared with those of Conn and Darrow, suggest that inability of certain soils to support growth of *Bact. globiforme* may be related to some special factor affecting productivity and not to general lack of crop-producing power.

The most recent study of the predominant micro-organisms in soils is that of Topping (36), who made an examination of the organisms which occurred most numerously in a series of soils from southeastern Scotland and Saxony. Gram-positive, non-spore-forming, non-acid-fast rod forms were found to outnumber all other types, as determined by a study of organisms growing

from the highest dilutions of soil on a variety of media. These Gram-positive bacteria were divided into three groups: (1) motile organisms producing branching variants, (2a) non-motile non-branching rods and (2b) non-motile, mycelium-forming types. All three groups represented forms exhibiting considerable pleomorphism. Organisms of Groups 1 and 2a particularly showed that the production of coccoid from rod forms closely resembled the morphological change undergone by *Bact. globiforme*, and the author considers strains of Group 2a to be probably related to this organism. This group is moreover believed to be related to *Corynebacterium*, while from their form members of Group 2b are considered to belong to Jensen's genus *Proactinomyces*. Though members of the motile Group 1 were not identified with known species the similarity in morphological and cultural behavior shown by strains of Groups 1 and 2 suggests a close relationship to the *Proactinomycetaceae*, motile species being recognized in both *Corynebacterium* and *Proactinomyces*. Gram-negative rods, classed in Group 3, were found to be less conspicuous than the Gram-positive forms. They were in the main chromogenic forms, and although they did not undergo the striking morphological changes exhibited by the Gram-positive types, they resembled the latter in their general biochemical inactivity.

In considering the evidence from qualitative studies so far reported a number of points seem to be established:

(1) In arable soils a large proportion of the bacterial flora consists of organisms whose functions are unknown or but little understood.

(2) Under normal soil conditions the predominant types of organisms are non-spore-forming short rods, motile or non-motile, cocci and spore-forming bacteria being relatively insignificant.

(3) Many predominant soil species are highly pleomorphic. Included in these are the *Bacterium globiforme* group and organisms closely related to corynebacteria and mycobacteria.

(4) The majority of soil bacteria are relatively inactive physiologically as judged by standard laboratory tests. This by no means excludes the possibility of important biological activity in soil.

The work so far done points to the value of more extensive studies of the qualitative nature of the soil microflora and the types predominating. Only when the autochthonous organisms are known more thoroughly will it be possible to learn their true functions in soil. Such knowledge is regarded as essential to an understanding of general microbiological activity in soil. In view of the intricate systems of symbiosis and antibiosis, it should help in evaluation of the known processes and form a basis for better appreciation of the relation of micro-organisms to growing plants, to soil borne plant diseases, and to soil fertility in general.

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QUALITATIVE STUDIES OF SOIL MICRO-ORGANISMS

II. A SURVEY OF THE BACTERIAL FLORA OF SOILS DIFFERING IN FERTILITY[1]

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Abstract

Investigations were made, on a non-selective basis, of the qualitative nature and relative incidence of the different types of the bacterial flora of three soils differing in fertility. The organisms were classified into eight groups. Non-spore-forming short rods, of which five groups were recognized, comprised nearly 90% of all types. Gram-negative short rods formed the most prevalent single group, rather more numerous than Gram-positive short rods. Gram-variable short rods, coccoid rods and pleomorphic rods *(Bact. globiforme)* were regarded as definite groups. Cocci, non-spore-forming long rods and spore-formers were less prominent soil types.

In spite of unequal productivity, the soils showed no outstanding differences in the relative incidence of the bacterial groups. Certain groups showed some indication of seasonal and cropping effect. The results suggest that the general character of the *autochthonous* (indigenous) soil flora is relatively uniform in soil of definite type, even though productivity may be greatly altered by manurial treatment.

The predominant soil bacteria appear relatively inactive in single culture. Moreover considerable divergence in biochemical action was shown by apparently closely related forms. It is suggested that the bacterial flora is relatively unstable physiologically, with considerable adaptability, and that the functions of the different species are exercised most fully only under conditions of association.

Introduction

The present paper is one of a number of studies on the qualitative nature of the microflora of soils, most of the relevant literature of which has been discussed in the first paper of this series (6). The object of the investigation was to study, on a non-selective basis, the bacterial types occurring in three soils, and their relative incidence. The soils were of similar type and crop history, but by reason of different fertilizer treatment for 25 years they had become widely dissimilar in productivity. A previous study (7) had been made of the abundance of certain strains of *Rhizobium* and *Azotobacter* in these soils, while as a side issue in the present work the incidence of *Bacterium globiforme* has been reported earlier (10).

Experimental

The soils studied were taken from three plots of different manurial treatment in a four-year rotation system of oats, clover, timothy and mangels. For the preceding 25 years the plots had been receiving the following treatments:

Soil N—no fertilizer

Soil X—15 tons farmyard manure, applied to mangels

Soil Y—100 lb. nitrate of soda, 300 lb. superphosphate, 75 lb. muriate of potash to mangels; 100 lb. nitrate of soda to oats, clover and timothy.

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The soils were of a sandy-loam nature and contained approximately 0.11%, 0.16% and 0.13% nitrogen respectively. The pH value fell between 7 and 8 with variations depending on crop, treatment and season. As may be noted from Table I, striking differences in fertility exist between treated and untreated plots. Mangel yields show this most clearly. The soils thus included an originally fertile soil impoverished by continuous cropping (N) and soils maintained at good fertility levels by farmyard and inorganic manures respectively (X and Y).

Composite samples were taken from the 2–4 in. layer, September 22, 1936, for preliminary tests, and on November 12, 1936, February 23, 1937, April 16, 1937, and July 21, 1937, from plots which had produced a crop of timothy and which at the time of the July sampling were supporting a mangel crop. In September and November, samples were likewise taken from corresponding plots after a crop of mangels. The February sampling represented frozen soil; but at the time of the April sampling the soil had thawed to a depth of 6 inches. Samples were analyzed as soon as possible. Plate cultures of 1/500,000 dilution were prepared. For comparison total cell counts were made by the ratio method of Thornton and Gray (12).

Cultural studies of the general microflora require a medium as non-selective as possible. For this reason, soil extract agar prepared according to Löhnis (8), and without added energy material, was chosen in preference to other media which, though synthetic and hence more easily reproducible, are regarded as more selective on account of the special energy sources contained. The advantage of soil extract was noted in previous tests (9), which gave higher counts with it than with more selective media such as Thornton's (11) mannitol-salts medium. From each sample five replicate plates were poured and incubated at 28° C. for 12 days before counting.

It is felt that the value of any qualitative study is enhanced when quantitative aspects are also taken into account. This is done not only by using definite dilutions, but by applying quantitative methods to an examination of the colonies. Haphazard selection or assumption of similar identity from microscopic observation are unsuited to an estimation of the relative incidence of different types. All colonies on a plate or on a definite sector should be examined on plates with a reasonable number of colonies. When few colonies are present the chances of error by regarding a possible contaminant as predominant are greatly increased. From representative plates all colonies on a sector containing approximately 60 bacterial colonies were picked and stab cultures made into soil extract semi-solid (containing 0.02% K_2HPO_4, 0.01% yeast extract and 0.3% agar). Preliminary tests indicated that many isolated strains, particularly from small colonies, refused to grow on various other media tested. The use of soil extract semi-solid not only assured the survival of 92% of the strains isolated but permitted a certain differentiation of type.

For group classification the cultures were examined for morphology and reaction to the Gram stain. To detect pleomorphic types of the *Bacterium*

globiforme group which appear first as rods and later change to cocci, observations were made on fresh transfers and on the same cultures at later stages of incubation. Standard agar was found to be an aid in the recognition of this group, though it was unable to support growth of many forms isolated. Physiological tests included gelatin liquefaction, nitrate reduction and dextrose utilization. For the nitrate reduction test semi-solid soil extract with 0.1% KNO_3 was used and for dextrose utilization soil extract semi-solid with 1% dextrose and indicator. The latter medium, being comparatively weakly buffered, was found to be more sensitive to changes than the usual media with peptone.

Plate and Total Cell Counts

A comparison of the three soils from the standpoint of "total numbers" is made in Table I in the form of summaries of the plate and cell counts. The usual seasonal fluctuation observed by so many previous workers is noted. The untreated soil appears less subject to fluctuation in numbers than the treated areas, particularly in cell counts. It is of interest to note that, although the plate count indicated that numbers were well maintained in the frozen (February) soil, cell counts showed in all three cases a drop from the November sampling. The results fail to show any relation between numbers and crop yield. In the case of the soils sampled after mangels particularly, there was little variation in numbers of organisms between the unfertilized and the fertilized areas, in spite of very large differences in crop-producing ability.

TABLE I

TOTAL CELL AND PLATE COUNTS

(millions per gram dry soil)

	Following timothy			Following mangels		
	N	X	Y	N	X	Y
Yield (tons per acre)						
av. 25 years	2.01	3.10	2.66	7.99	22.72	20.91
Yield in 1936	1.65	2.95	2.51	2.59	29.03	25.12
Total cell count						
September	741.2	990.1	2065.9	2403.6	2380.7	2332.6
November	1144.5	1846.8	2524.9	2241.4	2534.5	2166.0
February	982.3	1524.9	1018.5	—	—	—
April	681.3	1071.5	925.3	—	—	—
Plate counts						
September	57.0	52.2	50.8	76.6	109.2	107.9
November	92.6	95.4	116.8	123.1	139.1	126.4
February	90.0	111.3	132.7	—	—	—
April	72.4	81.1	60.0	—	—	—
July	36.3	60.1	70.6	—	—	—

Main Morphological Groups

From the morphological and Gram-staining reactions of the cultures in semi-solid soil extract eight main subdivisions of the isolated organisms were made:

Group I Short rods, Gram-positive
Group II Short rods, Gram-negative
Group III Short rods, Gram-variable
Group IV Short rods, changing to cocci (*Bact. globiforme* group)
Group V Coccoid rods, Gram-positive
Group VI Cocci, Gram-positive or negative
Group VII Long rods, non-spore-forming
Group VIII Spore-forming rods

In some instances difficulty was found in allocating organisms to groups, particularly in the case of certain Gram-positive short rods where the differentiation between rod and coccus was nearly impossible. Such types have been grouped as coccoid rods. Subsequent physiological tests provided some justification for the separate grouping of these forms. Short rods that later became cocci and conformed generally to *Bact. globiforme* Conn have been classified under this head. Short rods that showed no tendency to form cocci were divided into three groups according to their reaction to the Gram stain. The percentage distribution of the various groups in the three soils following timothy and sampled at four seasons is shown in Table II. The cultural characteristics of the various groups are summarized separately for the three soils and given in Tables III, IV and V.

Short Rods

In line with findings reported by a number of previous workers (1, 5, 13) non-spore-forming short rods were found to comprise a large proportion of organisms capable of being isolated from soils. The five groups into which short rods were classified made up 86.7%, 89.6%, and 89.1% respectively of cultures isolated from Soils N, X and Y, a surprisingly close agreement in soils differing so widely in productivity.

Gram-negative short rods. Gram-negative short rods were found to be the most prevalent single group of organisms, being rather more numerous than Gram-positive short rods in each soil, taken over the course of the four sampling periods. The difference was less pronounced in Soil N than in the fertilized soils X and Y. Variation in relative numbers was noted at different seasons. Topping (13) reported the Gram-negative group to be much less frequent than Gram-positive types in soils studied by her. As is seen in Tables III to V this group is the least active physiologically and is suppressed to the greatest extent by modifying soil extract through the addition of dextrose, 42 of 226 strains being entirely inhibited by 1% concentration. It is possible that differences in proportion of groups found may be due to employment of different media for isolation. Some of those employed by Topping may be

TABLE II

MAIN MORPHOLOGICAL GROUPS AT DIFFERENT SEASONS

(Soils following timothy)

	November			February			April			July		
	N	X	Y	N	X	Y	N	X	Y	N	X	Y
Soil moisture, %	15.7	17.2	18.1	24.1	32.3	26.2	24.9	23.1	26.1	13.5	15.9	17.6
Total cultures isolated	59	61	61	62	62	63	64	64	64	41	43	41
Percentage showing no growth on transfer	8.5	13.1	13.1	6.5	3.2	6.4	6.3	3.1	17.2	0.0	11.6	4.9
Percentage showing growth on transfer	91.5	86.9	86.9	93.5	96.8	93.6	93.7	96.9	82.8	100.0	88.4	95.1
Morphological groups	%	%	%	%	%	%	%	%	%	%	%	%
Short rods, Gram-positive	24.0	28.3	35.8	39.6	46.6	30.5	28.3	17.7	9.4	24.4	28.9	20.5
Short rods, Gram-negative	37.0	26.4	32.0	25.8	15.0	35.6	26.6	53.2	56.6	34.1	50.0	46.1
Short rods, Gram-variable	18.5	18.8	9.4	3.4	10.0	13.5	3.3	11.3	15.0	2.4	0.0	0.0
Short rods, changing to cocci (*Bact. globiforme* group)	12.9	9.4	11.3	8.6	10.0	6.7	10.0	8.0	7.5	14.6	0.0	7.7
Coccoid rods, Gram-positive	0.0	0.0	0.0	8.6	11.6	6.7	18.3	4.8	5.6	4.8	5.2	2.5
Cocci, Gram-positive or Gram-negative	3.7	3.7	5.6	10.3	3.3	1.7	6.6	4.8	1.9	0.0	0.0	0.0
Long rods, non-spore-forming	3.7	9.4	5.6	3.4	3.3	5.0	5.0	0.0	1.9	0.0	2.6	2.5
Rods, spore-forming	0.0	3.7	0.0	0.0	0.0	0.0	1.6	0.0	1.9	19.4	13.1	20.5

TABLE III

CHARACTERISTICS OF BACTERIAL GROUPS

(Soil N—no fertilizer)

Groups	Total no. of cult.	Per cent of total	Gr. on N. A. v. sl. or abs., %	Gelatine		NO_3 reduction, %	Soil-extr. s. s. + 1% dextrose				No action, gelatine, NO_3 or dextrose, %
				Growth, %	Liquef., %		No gr., %	Growth			
								Acid, %	Alk., %	No ch., %	
Short rods, Gram-positive	63	29.6	54.0	57.1	25.4	49.2	3.0	53.9	20.6	22.5	11.1
Short rods, Gram-negative	65	30.5	50.8	56.9	13.8	15.3	13.8	24.6	26.1	35.5	35.4
Short rods, Gram-variable	15	7.0	100.0	33.3	0.0	86.6	0.0	20.0	13.4	66.6	13.4
Short rods, changing to cocci (*Bact. globiforme* group)	24	11.2	0.0	100.0	100.0	33.3	0.0	75.0	16.6	8.4	0.0
Coccoid rods, Gram-positive	18	8.4	88.8	38.8	16.6	61.1	11.1	38.8	27.7	22.4	16.6
Cocci, Gram-positive or negative	12	5.6	91.6	41.6	33.3	66.6	8.4	41.6	25.0	25.0	0.0
Long rods, non-spore-forming	7	3.3	57.1	85.7	14.3	14.3	14.3	28.5	28.5	28.7	42.9
Rods, spore-forming	9	4.2	11.1	66.6	44.4	44.4	11.1	66.6	0.0	22.3	22.3

TABLE IV

CHARACTERISTICS OF BACTERIAL GROUPS

(Soil X—farmyard manure)

Groups	Total no. of cult.	Per cent of total	Gr. on N. A. v. sl. or abs., %	Gelatine		NO_3 reduction, %	Soil-extr. s. s. + 1% dextrose				No action, gelatine, NO_3 or dextrose, %
				Growth, %	Liquef., %		No gr., %	Growth			
								Acid, %	Alk., %	No ch., %	
Short rods, Gram-positive	65	30.5	72.3	38.5	16.9	60.0	16.9	46.1	10.8	26.2	16.9
Short rods, Gram-negative	75	35.2	49.4	40.0	17.3	12.0	17.3	25.3	24.0	33.4	38.5
Short rods, Gram-variable	23	10.8	100.0	40.0	17.4	82.5	4.3	34.8	13.0	47.9	8.7
Short rods, changing to cocci (*Bact. globiforme* group)	16	7.5	0.0	100.0	100.0	25.0	0.0	75.0	12.5	12.5	0.0
Coccoid rods, Gram-positive	12	5.6	83.3	33.3	33.3	75.0	8.3	50.0	16.7	25.0	8.3
Cocci, Gram-positive or negative	7	3.3	100.0	57.1	14.3	85.7	0.0	42.8	14.3	42.9	0.0
Long rods, non-spore-forming	8	3.8	12.5	87.5	50.0	12.5	12.5	12.5	25.0	50.0	25.0
Rods, spore-forming	7	3.2	0.0	85.7	85.7	85.7	0.0	42.8	14.2	43.0	0.0

TABLE V

CHARACTERISTICS OF BACTERIAL GROUPS

(Soil Y—mineral fertilizers)

Groups	Total no. of cult.	Per cent of total	Gr. on N. A. v. sl. or abs., %	Gelatine		NO_3 reduction, %	Soil-extr. s. s. + 1% dextrose				No action, gelatine, NO_3 or dextrose, %
				Growth, %	Liquef., %		No gr., %	Growth			
								Acid, %	Alk., %	No ch., %	
Short rods, Gram-positive	50	24.5	84.0	48.0	18.0	48.0	14.0	46.0	10.0	30.0	30.0
Short rods, Gram-negative	86	42.1	64.0	48.6	19.7	5.0	23.2	37.0	19.7	20.1	38.3
Short rods, Gram-variable	21	10.3	100.0	47.6	33.3	66.6	4.8	42.8	23.8	28.6	4.8
Short rods, changing to cocci (*Bact. globiforme* group)	17	8.3	0.0	100.0	100.0	52.9	0.0	82.3	0.0	17.7	0.0
Coccoid rods, Gram-positive	8	3.9	100.0	50.0	25.0	87.5	0.0	50.0	12.5	37.5	0.0
Cocci, Gram-positive or negative	5	2.4	80.0	40.0	20.0	20.0	20.0	0.0	20.0	80.0	60.0
Long rods, non-spore-forming	9	4.4	33.4	55.5	11.1	11.1	22.2	33.3	22.2	22.7	22.2
Rods, spore-forming	8	3.9	0.0	100.0	75.0	25.0	12.5	50.0	12.5	25.0	12.5

considered as fairly selective while there is no indication of what proportions of the organisms studied by her originated on the several media used.

Gram-positive short rods. Gram-positive short rods were the second most abundant group in all soils. This group displayed rather more activity than Gram-negative forms as judged by ability to reduce nitrates, liquefy gelatine or utilize dextrose. Like the latter group, however, a considerable percentage showed no growth on ordinary gelatine or agar and are believed to represent largely forms indigenous to soil only, brought out by such media as soil extract.

Gram-variable short rods. Gram-variable short rods appeared to comprise a definite group failing to give a uniform reaction to Gram staining, though both Hucker's and Kopeloff's modifications were used. Though numerically less important than either of the above groups they showed certain characteristics which presumably justified their being classified separately. None of 59 strains isolated was able to grow on nutrient agar while their most pronounced biochemical feature was their comparatively high nitrate-reducing ability.

Cocci-forming rods. Cocci-forming rods classified as the *Bacterium globiforme* group were an important group in all soils studied, comprising 11.2%, 7.5% and 8.3% of the organisms isolated from Soils N, X and Y respectively. Members of this group, though definite rod forms in young cultures, show a change to the coccal form with longer incubation. As previously indicated (10) variations in cell size and rate of change from rod to coccus are noted between different strains of this group. Physiological tests further emphasized differences which may be exhibited by apparently closely related strains. Thus in a separate experiment in which 50 cultures of *Bact. globiforme* were compared as to ability to utilize six sugars, hydrolyze starch and reduce nitrates, 40 actual variations in physiology were noted. As a group these organisms were the most active of those found, and were uniform in ability to liquefy gelatine and grow on standard media.

Coccoid rods. Coccoid rods, representing Gram-positive short rods that could not be satisfactorily differentiated from cocci, produced 8.4%, 5.6%, and 3.9% of the organisms isolated from Soils N, X, and Y. Like the Gram-variable short rods, the great majority failed to grow on nutrient agar, and included a large proportion of nitrate-reducing forms.

Cocci, Long Rods and Spore-formers

These three groups represent less prominent soil bacteria, judging from their abundance in the soils studied. Cocci comprised 5.6%, 3.3% and 2.4% of the strains isolated from the three soils. Conn (1, 2) found cocci to be numerically insignificant, and inclined to the belief that they are not to be regarded as characteristic of soil. In the soils studied by us the cocci isolated showed much variation in type and appeared to represent a variety of species each present in but small numbers.

Non-spore-forming long rods. This group comprised 3.3%, 3.8% and 4.4% of the total organisms isolated from the three soils, while spore-forming

rods were found to the extent of 4.2%, 3.2% and 3.9%. Only in the case of the July sampling did the last-named group represent an appreciable percentage of the forms isolated.

Discussion of Predominant Forms

A close comparison with the short rod forms described by Conn (2, 3) and Topping (13) is made difficult on account of the use of different media, making for differences in grouping and in estimating relative abundance in soil. Thus Gram-negative rods were of greater significance than is indicated by the work of these authors. However it appears that the non-spore-forming short rods comprising our Groups I to V correspond in large measure to types of short rods described by Conn and particularly to those classed as "slow growers" or "punctiform colony forming" organisms. Such terms may be misleading in some instances, since organisms producing little growth on a comparatively deficient medium, such as tap-water gelatine, may grow profusely on other media. This has been found to be the case with organisms included in our *Bact. globiforme* Group IV.

It appears that Conn's Group I, comprising simple rods, includes types which we have subdivided into our Groups I, II, III, and V. On the other hand our *Bact. globiforme* group is rather more inclusive than Conn's, embracing not only his Group II *(Bact. globiforme)*, comprising forms showing change from rod to coccus, but also his less abundant Groups III and IV, characterized by a tendency to produce branched forms. The last-named groups are doubtless related to pleomorphic, coccus-forming types forming "sprouts", or branching forms described by Topping, who suggests a close relationship between her Groups 1 and 2 and *Bact. globiforme*. Belief in this relationship is strengthened by observation of strains of *Bact. globiforme* including one obtained from Dr. Conn which show ability, especially in liquid media, to produce branching forms characteristic of Conn's Groups III and IV and Topping's pleomorphic groups. Moreover comparison of Figs. 11, 5, 3 and 15 given in Topping's paper with Figs. 1, 2, 3 and 5 respectively in the paper of Taylor and Lochhead (10) on *Bact. globiforme* suggests that these authors worked with very closely related forms. The apparently higher incidence of pleomorphic types comprising *Bact. globiforme* and related forms found by Conn and Topping as compared with our present studies is believed to be due largely to the use of media more selective for these forms. The selective action of tap-water gelatine as compared with soil extract agar has been previously demonstrated (10).

Relation of Bacterial Groups to Soils Studied

In Table II, giving the incidence of the different morphological groups in the three soils at four sampling dates, no outstanding differences are seen between the unfertilized soil, N, and the fertilized soils, X and Y, in spite of great variation in crop-producing capacity. The uniformity is the more interesting since Soil X received an application of farmyard manure three

weeks previous to the November sampling. Slightly higher percentages of non-spore-forming long rods and of spore-formers were noted in this soil but otherwise the group incidence approximated closely that of Soils N and Y. In line with the findings of Joffe and Conn (4) it is apparent that if notable increases in spore-formers are to result from addition of organic materials, amounts in excess of normal field applications are needed.

Throughout the tests there was no indication that the incidence of *Bact. globiforme* bore any relationship to the productivity of the soils examined, nor was it possible to correlate fertility with the relative abundance of any of the other main groups into which the organisms were classified. The soils in question, originally the same, had become different in crop-producing ability by artificial means, and the results suggest that in a soil of given type the bacterial flora may be fairly resistant to change, even though productivity may vary as much as ten-fold.

There is indication of rather more difference in group incidence due to cropping than between the soils themselves. Comparative data, following timothy and mangels respectively, are given in Table VI, in which the approximate numbers in millions per gram dry soil are shown. After mangels, increased counts of Gram-positive and Gram-variable short rods and *Bact. globiforme* were found, as compared with the numbers following timothy. Mangels in general supported a higher bacterial population as measured by both total and plate counts (Table I).

TABLE VI

BACTERIAL GROUPS IN THREE SOILS FOLLOWING DIFFERENT CROPS

Morphological group	Millions per gram of dry soil					
	Following timothy			Following mangels		
	N	X	Y	N	X	Y
Short rods, Gram-positive	20.0	22.3	34.6	28.7	55.7	44.1
Short rods, Gram-negative	30.8	20.9	30.9	16.4	23.2	27.3
Short rods, Gram-variable	15.4	14.9	9.1	24.6	16.2	14.7
Short rods, changing to cocci (*Bact. globiforme* group)	10.7	7.4	9.1	18.4	13.9	14.7
Cocci, Gram-pos. and Gram-neg.	3.1	3.0	5.5	8.2	0.0	12.6
Long rods, non-spore-forming	3.1	7.4	5.5	8.2	6.9	2.1
Rods, spore-forming	0.0	3.0	0.0	4.2	0.0	0.0

The effect of season on the relative incidence of the different bacterial groups was in general not marked (Table II). The most pronounced changes occurred in the July sampling, which showed a decrease in Gram-variable short rods and a rather notable increase in spore-formers in all soils. At this sampling the mangel crop was growing but no definite reason for the degree of prominence of this latter group is offered.

Conclusion

Micro-organisms in the untreated soil, N, might be expected to represent for the most part the *autochthonous* microflora, *i.e.*, the indigenous group of

organisms postulated by Winogradsky (14, 15) as contrasted with the *zymogenic* group, composed of forms relatively scarce in normal soils, but becoming active upon additions of readily decomposable substances. This latter group might be supposed to be more evident in Soil X, receiving farmyard manure. As judged from the grouping of the bacteria isolated, however, no evidence of alteration of types was found, suggesting that the autochthonous flora of the soil type studied was little affected by the treatments given. Whether differences exist that are not brought out by general grouping, would have to be decided by studies of a much more specific nature.

More detailed study of strains isolated, however, particularly physiological tests, showed a surprising variability in apparently very closely related types. In each of the main groups identity of characteristics was the exception rather than the rule, with each additional test bringing out slight divergencies. The degree of biochemical activity displayed by the predominant soil organisms was regarded as relatively low. It is suggested that the indigenous bacterial flora is comparatively unstable physiologically and possessed of considerable adaptability. The comparative inactivity of so many forms when isolated from soil and cultivated singly also suggests that the functions of these species are exercised most fully only when they are acting in association with other micro-organisms. In aiding toward a better understanding of these functions research with mixed cultures will doubtless play an important part. The limitations attendant on an investigat. n of one type of soil, as in the present study, are recognized, and it is hoped to extend the work to a variety of fertile and infertile soils of different types.

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THE TRANSFERENCE OF DURUM AND DICOCCUM CHARACTERS TO 21-CHROMOSOME WHEAT LINES BY CROSSING[1]

By T. J. Arnason[2]

Abstract

Hybrids between *vulgare* ($n = 21$) and emmer ($n = 14$) wheats were backcrossed to *vulgare*, and segregates having the *vulgare* chromosome number were studied with respect to their emmer characters. Eleven of the 24 characters used in a *vulgare-durum* cross and 6 of the 23 characters used in a *vulgare-dicoccum* cross appeared in emmer condition in hybrids. Most of the other characters differed from the *vulgare* form in a number of segregates. From a comparison of the results of this investigation and a previous one dealing with 14-chromosome segregates, it is concluded that the *vulgare* form of many characters is due to genes in the primary chromosome sets A and B, the *vulgare* form of many others to genes in both the primary sets and the secondary set C.

Introduction

The transmission of some *vulgare* characters to stable 14-chromosome* segregates was discussed in a paper by Thompson, Arnason and Love (1). The account that follows deals with the transfer of *durum* or *dicoccum* characters to 21-chromosome lines derived from *vulgare-durum* and *vulgare-dicoccum* crosses.

Twenty-one-chromosome segregates of a *vulgare-durum* or *vulgare-dicoccum* cross presumably can have any combination of *vulgare* and *durum* or *dicoccum* chromosomes from the primary sets A and B which mate in F_1 together with the complete C set of *vulgare* which lack mates in F_1. By repeated selfing in several lines many plants homozygous for a few *durum* or *dicoccum* genes should be obtained. Observations on large numbers of plants from many lines should show then whether the genes from emmer series plants can produce effects in 21-chromosome plants similar to those they produce in 14-chromosome ones. When such an effect is obtained, *i.e.*, when a character appears in *durum* or *dicoccum* condition, the *vulgare* genes in the C set have not apparently affected its expression. If the character in question has been reported in *vulgare* form in 14-chromosome plants it may then be concluded that no genes of importance affecting the character are present in the C set. If the *vulgare* form has not appeared in 14-chromosome plants, its production probably depends on interaction between genes in the primary sets A or B with C-set genes or upon multiple factors, some of which are in the C set.

Materials and Methods

The parental strains were Marquis (V), a *vulgare* wheat, Iumillo (D), a *durum*, and Vernal, (E), a *dicoccum*. VD and VE plants, F_1 generation, were backcrossed to Marquis. The backcross (F_2) plants (V-VD and V-VE)

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* *Haploid chromosome numbers are used throughout.*

were selfed, as were their progeny. Some backcross lines have been carried to F_6, others to F_4 or F_5 only. A number of stable lines ($n = 21$) have been obtained, a few lines are still unstable and a few have reverted to the 14-chromosome condition.

Chromosome counts were made on pollen mother cells from a total of 294 F_4, F_5, and F_6 plants. Most of these counts were made on smeared material, but some were made from permanent slides fixed in Karpechenko's modification of Navashin's fixative, and stained with crystal violet and iodine. The complete count could not always be made. In such cases where many cells had no univalents, the plants were interpreted as having 21 bivalents. Occasionally rings of four chromosomes or chains of three were encountered, but these were rare.

Characters Studied

The backcross V-VD plants were examined to determine the condition of 24 morphological characters; the V-VE plants were examined for the condition of 22 characters. Brief descriptions of the characters and their condition in the parental varieties are given below. The descriptions are based in each case on about 100 observations or measurements.

NOTE: v = *vulgare*, d = *durum*, e = *dicoccum*

1. Stem diameter—measured at 2 cm. below the collar; v, 1.6–2.5 mm.; d, 1.1–1.6 mm.; e, 1.1–1.5 mm.
2. Stem cavity—observed 2 cm. below collar; v, large cavity, thin walls; d and e, no cavity.
3. Collar—at base of spike; v, $\frac{3}{4}$ of stems have open collar; d, all closed; e, $\frac{1}{8}$ open.
4. Head length—measured from the base of the lowest spikelet to the tip of the terminal one; v, 7.5–11.0 cm.; d, 5.5–7.5 cm.; e, 6.1–8.4 cm.
5. Density of spike—calculated by dividing the head length by the spikelet number; v, 4.7–6.3; d, 3.5–4.7; e, 3.6–4.7.
6. Head form—width of the 1-ranked side divided by the width of the 2-ranked side at the centre of the spike; v, 1.2–1.6; d, 0.8–1.1; e, 0.5–0.8.
7. Glume adherence; v and d, loose; e, tight.
8. Glume length, exclusive of glume tooth; v, 7.8–9.0 mm.; d, 9.0–10.3 mm.; e, 9.1–11.0 mm.
9. Glume shape—empty glume; v, wide at top and bottom, broadest near base, blunt; e and d, broadest near the middle, tapering to both ends.
10. Glume cross-section taken near the centre of the glume; v, broad U-shaped; d, V-shaped; e, nearly V-shaped.
11. Keel prominence; v, prominent but not deep and sharp as in d and e.
12. Keel hairs; v, sparse stout hairs on upper half of keel, fine or none on lower half; d, very fine, closely placed hairs from tip to near base; e, few, usually about 10, stout hairs near the tip.

13. Glume tooth; v and e, short terminal tooth 1 mm. or less in length; d, long tooth, 2–3 mm.
14. Tooth sharpness; v, blunt; d, sharp, fine point; e, rather dull, coarse point.
15. Beard length—measured on middle spikelets; v, up to 1 cm.; d, 10–12 cm.; e, 10–13 cm.
16. Glume shoulder; v, wide, nearly horizontal; d and e, narrow, sloping down sharply.
17. Rachis width—1—measured on central segments at widest point; v, 2.5–3.2 mm.; d, 2.0–2.4 mm.; e, 1.1–1.8 mm.
18. Rachis width—2—measured at the narrow end. In all cases the lower end is the narrow end; v, 1.7–2.3 mm.; d, 1.3–1.6 mm.; e, 0.7–1.3 mm.
19. Rachis shape; v, curving, widest point a short distance below the top of the segment; d and e, sides straight, segment widest at the top.
20. Rachis hair tuft—the hairs at the centre of the rachis face, between the bases of the empty glumes; v, few, sparse, rather short; d, single large tuft of long hairs; e, small compact tuft of long hairs.
21. Rachis hairs along sides; v, long hairs uniformly and rather sparsely distributed from top to bottom; d, fine hairs closely placed, long at top, short near base of rachis segment; e, sides bare.
22. Rachis hairs in upper corners; v, few long hairs; d, dense tuft of long hairs; e, none.
23. Rachis fringe—hairs along the top of the rachis segments (except the central tuft); v, few short scattered hairs; d and e, none.
24. Rachis articulation; v and d, tough; e, brittle, breaks at base of rachis segments.
25. Grain length; v, 5.0–6.2 mm.; d, 6.0–7.5 mm.; e, 7.0–9.0 mm.
26. Grain hairs; v and e, long, many; d, short, few.
27. Leaf hairs—upper side of young leaves; v, sparse, moderately long hairs at crests of ridges, few short hairs on sides; d, no hairs; e, many long hairs uniformly distributed.

Durum and *Dicoccum* Characters Appearing in 21-chromosome Segregates

Pollen mother cells of 198 V-VD plants representing 12 lines, and 96 V-VE plants representing 7 lines were examined. Of these, 86 V-VD and 22 V-VE plants, representing 9 and 3 lines respectively, had 21 pairs of chromosomes. As used here a line includes all the descendents of an F_3 (*i.e.*, a second generation backcross) plant. The *durum* and *dicoccum* characters which have appeared apparently unchanged in 21-chromosome plants are listed in Table I. It will be observed that fewer *dicoccum* than *durum* characters were transferred to 21-chromosome plants. This may be due, in part, to the fact that fewer 21-chromosome *dicoccum* hybrids were found. The smaller number of these was due to the fact that in *dicoccum* hybrids cytological irregularities were

TABLE I

CHARACTERS FOUND IN *durum* OR *dicoccum* CONDITION IN 21-CHROMOSOME SEGREGATES

Character	*Vulgare-durum* hybrids		*Vulgare-dicoccum* hybrids	
	No. of plants	No. of lines represented	No. of plants	No. of lines represented
Stem diameter	5	3		
Collar	5*	4		
Head length	2	2		
Head form	21	8	2	1
Glume length	11	5		
Glume tooth	7	3		
Tooth sharpness	29	4		
Shoulder	13	4	5	2
Grain length	17	9	2	2
Rachis fringe	36	6	6	3
Rachis hairs in upper corners	12	4		
Glume adherence			4	2
Rachis hair tuft			6	2

* *Failed to breed true.*

eliminated with greater difficulty than in *durum* hybrids. Some of the differences in the results from the two crosses may be attributed to genetic differences between *durum* and *dicoccum*. This is obvious in cases in which one of the emmer species resembles *vulgare* with respect to a character, while the other species differs, *e.g.*, the rachis articulation character.

Characters which failed to appear in completely emmer condition but did appear in intermediate condition are listed in Table II. Eight characters

TABLE II

CHARACTERS FOUND IN INTERMEDIATE CONDITION IN 21-CHROMOSOME PLANTS

Character	*Vulgare-durum* hybrids		*Vulgare-dicoccum* hybrids	
	No. of plants	No. of lines represented	No. of plants	No. of lines represented
Cavity of stem	8	3	1	1
Density	1*	1		
Glume cross-section	3	3	1	1
Glume shape	32	7		
Keel prominence	24	5		
Keel hairs	18	5		
Beard length	30	6	14	3
Rachis width-1	3†	2		
Rachis width-2	2†	2		
Rachis curve	13†	4	12†	4
Rachis hair tuft	3	3		
Grain hairs	12	3		
Rachis articulation			8	4

* *Progeny all had v density.*

† *Shift from vulgare very slight.*

appeared in intermediate condition in *vulgare-durum* hybrids; four in *vulgare-dicoccum* hybrids. The rachis width and curve characters mentioned in the table were very near *vulgare* in all cases and the *durum* density recorded for one plant may have been brought about by environmental factors, since the character did not appear in any of the progeny of this plant.

Certain characters appeared in exaggerated *vulgare* form in some segregates. These V+ or super-*vulgare* characters are listed in Table III. Five of these were in *vulgare-durum*, three in *vulgare-dicoccum* hybrids.

TABLE III

V+ CHARACTERS IN 21-CHROMOSOME LINES

Character	*Vulgare-durum* hybrids		*Vulgare-dicoccum* hybrids	
	No. of plants	No. of lines represented	No. of plants	No. of lines represented
Head length	12	4	3	1
Density of spike	18	7	9	3
Head form	11	5	2	1
Rachis width-1	5	3		
Rachis width-2	1	1		

The Mode of Inheritance of Some Characters

Some of the *vulgare* characters that have been observed in 14-chromosome segregates (1) have their *durum* and *dicoccum* counterparts in 21-chromosome plants. Such characters are stem diameter, glume length, tooth length, tooth sharpness and glume adherence. All the genes necessary for the production of the *vulgare* form of these characters must lie in the A and B sets of chromosomes. A number of other characters such as head length, glume shoulder, rachis fringe, rachis hair tuft and seed length may appear in *durum* or *dicoccum* condition in 21-chromosome plants, but it is doubtful whether the *vulgare* condition can be obtained in 14-chromosome plants. It is possible that in such cases *durum* and *dicoccum* genes are epistatic to certain genes in the *vulgare* C set. This may be illustrated by the observation made in the case of glume shoulder characters. No 14-chromosome plant was credited with a completely *vulgare* glume shoulder, though some approached this form. The *durum* condition occurred in 21-chromosome plants. Hence it appears likely that *vulgare* has genes influencing the shape of the glume shoulder in the primary sets (A or B) and in the secondary set (C). *Durum* genes must then be epistatic to the *vulgare* C genes.

The character called head form may be affected in several ways, for example by glume length, glume width, glume adherence and the plumpness of the seeds. Since a numerical ratio of width to depth only is taken, plants listed as having similar head form are not necessarily genetically alike with respect to the character, as similar ratios may be brought about by different combinations of glume and other spike characters. Also, since the character is

expressed in terms of a ratio, the absolute measurements may differ from those of the parent which has a similar head form. As might be expected, some factors affecting head form are believed to lie in the C set of chromosomes in spite of the fact that the *durum* and *dicoccum* forms do appear in 21-chromosome segregates. In 14-chromosome derivatives of a *vulgare-dicoccum* cross *vulgare* head form was not observed (1). All the 21-chromosome plants having *dicoccum*-type head form also had tightly adhering glumes and long rachis segments.

Characters which never appeared in *durum* or *dicoccum* condition in 21-chromosome segregates and which are, therefore, presumed to be conditioned by C-set genes, include the following: stem cavity, density of spike, rachis width, rachis shape, rachis articulation (toughness), glume shape, glume cross-section, keel prominence, keel hairs, beard length, rachis hair tuft, rachis hair sides, leaf hairs. Some of these, and one additional character, will be dealt with individually.

In the *durum* variety used all the collars were closed. The *vulgare* variety was less constant. Usually in a plant having several tillers most of the collars were open, but often a few were closed. A count of 100 *vulgare* stems gave a ratio of three-fourths open to one-fourth closed. Some of the 21-chromosome hybrid segregates had a smaller proportion (one-half) of open collars than had *vulgare*. A few plants with all the collars closed were found but in every case each of the progeny had some open collars. The conclusion arrived at in an earlier paper (1) that the open collar of *vulgare* is dependent on C-set genes may be correct but it appears that genic control of this character is not complete.

Previous work (1) has shown that the near-beardless condition of *vulgare* is due to genes in the primary chromosome sets. Many 21-chromosome segregates had long beards but not as long as in the *durum* or *dicoccum* parents (1 to 4 cm. shorter). Therefore, it is concluded that in the C set one or more beard-modifying factors are present.

While no 21-chromosome derivative of the *vulgare-dicoccum* cross had as brittle a rachis as *dicoccum*, many were more brittle than *vulgare*. In addition, some segregates having less than 21 bivalents were more *dicoccum*-like in that respect, confirming the view that genes for tough rachis are present in the *vulgare* C set.

Vulgare has long sparse hairs on the ridge tops of the leaves and few short hairs on the ridge sides. *Durum* has no hairs and *dicoccum* has many long hairs uniformly distributed on the ridge tops and sides. All the 21-chromosome hybrids from both crosses had long ridge-top hairs though there was some variation in their number and length. In the *dicoccum* cross long hairs also occurred often on the ridge sides. These side hairs were not as long as some top hairs. In *durum* hybrids long hairs never occurred on the ridge sides, and the top hairs, though classed as long, were considerably shorter than in *dicoccum* hybrids. *Vulgare*-type leaf hairs were previously reported as occurring in several 14-chromosome *vulgare-dicoccum* hybrids and one *vulgare-*

durum hybrid (1). The facts suggest that two or more genes affecting leaf hairs are present in *vulgare*. Probably at least one gene influencing especially ridge-top hairs lies in the C set.

The inheritance of rachis hair characters is not clear. Since many 21-chromosome segregates had no fringe across the top, it is concluded that genes for fringe hairs are lacking from the C set. The *vulgare* type of rachis hairs at the sides occurred in all *vulgare-dicoccum* segregates. A gene for these hairs is probably in the C set; another such gene is then probably in A or B since "near-*vulgare*" rachis hairs were reported for some 14-chromosome segregates (1).

The exaggerated *vulgare* characters in the V+ group must depend in part on the genes of the C set of chromosomes, since these characters never appear in 14-chromosome segregates. The V+ characters may be due to multiple factors, to interaction between *durum* or *dicoccum* and C set genes, to gene duplication or to polysomy. In the absence of critical cytological evidence a decision cannot be made between the alternatives.

The sharp tooth of the empty glume, a characteristic of the *durum* variety, appeared in a number of hybrids. In other segregates the tooth was extended to form a short awn. Similar short awns were observed also in 14-chromosome segregates (1). The awned condition was always associated with beards though the shorter *durum*-type tooth appeared occasionally in beardless plants. Watkins has reported linkage between beards and glume-tooth awns (2). The absence of awns in the bearded parental varieties must be due to awn-suppressing genes. The awned segregates lack the suppressors. There is no evidence that C-set genes affect the expression of this character.

Discussion

The *vulgare* form of some characters did not appear in any of the 14-chromosome segregates examined by Thompson, Arnason and Love (1). Of this group of characters, those which are always *vulgare* in 21-chromosome segregates, *e.g.*, the rachis width characters, must owe that form to C-set genes. However, the *vulgare* form of some of these characters, *e.g.*, head form, does not occur in all 21-chromosome segregates. For such characters C-set genes, together with certain A- or B-set genes, are necessary.

In one case it has been shown that the genes necessary to produce the *vulgare* form are not all the genes that may affect the character. The *vulgare* beardless condition is easily transmitted to 14-chromosome segregates, but the long beards of emmer were not found unmodified in 21-chromosome plants. Presumably beard-modifying factors are present in the C set. In normal beardless *vulgare* these factors would have no visible effect.

In general, conclusions arrived at earlier, with respect to the location of genes responsible for *vulgare* characters, are supported by the results reported here. The present study has shown also some of the variations of form that may be brought about by shuffling together primary-set genes of emmer and *vulgare* and dealing out new "hands", including in each a complete C set.

If, as seems probable, *vulgare* wheat is an allopolyploid, in which the C set of chromosomes came from a plant whose haploid chromosome number was 7, then originally this set must have contained genes affecting all parts of the plant. How far the integrity of this or any set of chromosomes has been maintained is not known. It is not unlikely that some rearrangement of chromatin has taken place both within and between chromosomes.

Doubtless the original allopolyploids had many of the characters, or combinations of characters, that occur in modern members of the *vulgare* group; other character modifications probably appeared as a result of gene mutations and gene losses. But these presumably would be equally likely to occur in any chromosome. For this reason differentiating factors might be expected to be scattered, not confined to any one "set" of chromosomes. This appears to be the case in our material. The *vulgare* form of some structures is determined mainly by genes in the A and B sets, of others by genes in the C set, still others require genes from the primary sets (A and B) and the secondary set (C) for the production of the typical *vulgare* form.

Characters which are confined to 21-chromosome lines of wheat must depend on genes in the 7 chromosomes of *vulgare* which do not pair with members of the "emmer series" chromosomes in hybrids. All others can appear in 14-chromosome plants. The known number of "group distinguishing" characters is quite small. Most *vulgare* characters are not distinguishing, in the sense that they do not occur in any 14-chromosome plants (2).

Conclusions

The primary sets A and B, of Marquis wheat *(vulgare)* differ in many genes from those of Iumillo *(durum)* and Vernal *(dicoccum)*. Some of these *vulgare* genes produce typical *vulgare* characters in 14-chromosome plants, but some do not. Similarly some of the "emmer series" genes produce typical "emmer" characters in 21-chromosome plants, while others do not.

Genes of the C set affect many characters, and must affect all those which can not be obtained in *vulgare* form in any 14-chromosome segregates. The many "emmer series" characters that fail to appear unchanged in 21-chromosome plants must owe their modification in many cases to C-set genes. It is quite possible that more of these characters than have been reported here can be transferred unchanged. This is especially true of derivatives of the *vulgare-dicoccum* cross in which the group of determined 21-chromosome plants was very small.

Acknowledgments

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STUDIES ON BLACKHEART, SOFT-ROT, AND TARNISHED PLANT BUG INJURY OF CELERY[1]

By J. K. Richardson[2]

Abstract

A study of blackheart, soft-rot and tarnished plant bug injury on celery was made to determine their interrelationship, in addition to their individual effects on the host. The incidence of blackheart could not be correlated with a saturated condition of the soil, or with the use of various fertilizers, but under artificial conditions the disease could be induced in susceptible plants by subjecting them to temperatures ranging from 85–95° F., in a humid atmosphere. Experimental data supplemented by observations in the field indicate (i) that the disease is physiological in nature, (ii) that early plantings are more severely affected, (iii) that most extensive injury occurs when plants are nearing maturity, (iv) that vigorous plants are more subject to attack, (v) that there is a difference in varietal susceptibility, and (vi) that the appearance of the disease in the field is generally preceded by a period of high humidity or of high temperature, or of both.

In addition to the soft-rot caused solely by *Erwinia carotovora* (L. R. Jones) Holland, necrotic blackheart tissues under favorable conditions often become infected by this pathogen, which, as a secondary decay, destroys the plant.

The tarnished plant bug *Lygus pratensis* L. is of economic importance as a vector of soft-rot. Considerable damage, differing in appearance from either blackheart or soft-rot, may also be caused by its feeding habits.

Introduction

In some seasons, blackheart is extremely prevalent and destructive in certain districts of Ontario. Much of the celery in the province is grown in the market-gardening districts where a variety of soil types and cultural practices obtain. Most growers in these localities have suffered severe losses from this disease, but when questioned, could supply only meagre information concerning the trouble.

The disease has been attributed to unsuitable environmental conditions, infection by *Erwinia carotovora*, attacks by certain insects, or a combination of two or more of these factors. The present studies were undertaken to investigate the relative importance and possible interrelations of the various factors involved in blackheart of celery in Ontario.

Review of Literature

The first reference to a heart rot of celery was made by Halstead in 1892, when he described a bacterial disease occurring in New Jersey, which closely resembled soft-rot of carrots. The following year Beach (2) described two troubles present in New York State, one of which was a typical soft-rot, the other a withering of the leaflets and decay of the stalks, although both conditions may have been symptoms of the same disease. The cause of the trouble was not determined, but it was more prevalent during the summer months and progressed most rapidly when conditions were moist and hot.

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In a favorable environment, however, plants that were not too badly attacked were able to form new hearts. Kinney (9) reporting a similar disease in Rhode Island in 1897 named it "blackheart", stated that it was widespread and not confined to any particular varieties, that it was not seed-borne, that it occurred on both irrigated and non-irrigated land, and that it appeared to be worse after periods of high temperature. Winters (16) in Florida in 1907 and 1908, conducted extensive research on blackheart and came to the conclusions, (i) "that some physiological weakness produced bleaching, which made the plants susceptible to infection by bacteria; (ii) that certain fertilizers increased the susceptibility of plants to the disease; (iii) that bacteria were not the direct cause of blackheart." In 1915 (1) a disease similar to that occurring in Florida was reported from Bermuda. In England in 1914 and 1916, Wormald (17, 18) gave a complete description of soft-rot of celery caused by a strain of *E. carotovora*. Poole (13) stated that in New Jersey investigations were being carried on with stem and root rot of celery in 1917, and in 1921 (14) he reported severe losses due to bacteriosis in the same state. In the same year Smith (15) reported that in the delta region in California, a celery crown rot was found to be due to the soft-rot organism *E. carotovora*. Foster and Weber (5) in 1924 discovered that the blackheart prevalent in Florida was not influenced by fertilizers, but could be induced at will by flooding plants by means of sub-irrigation. In 1932, Foster (4), reporting the results from a questionnaire distributed throughout the United States and Canada, states that while blackheart is sometimes confused with bacterial soft-rot, the latter causes a decay of the affected parts without hope of recovery, whereas the former, which originates in the heart of the plant, is frequently outgrown.

The relation of insects to blackheart and soft-rot has received considerable attention. In 1917 Hearst (7) reported a rapid bacterial heart decay of celery plants which was associated with, and secondary to, the injury of young shoots caused by the insect *Lygus pratensis* L. Ten years later Leach (10) described a bacterial heart rot which was spread in the field by larvae of the dipterous leaf miners *Scaptormyza gramineum* Fall., and *Elachiptera costata* Leow. In England in 1934, Ogilvie (11) associated soft-rot with the injury of carrot flies, slugs and the drosophilid, *S. gramineum* Fall.; and in Ontario Caesar (3) reported that the insect *L. pratensis* L. kills the tips of the inner leaf stems of the celery plant, thus enabling bacteria to enter and destroy the heart.

Blackheart

Symptoms of the Disease

The initial symptoms of blackheart develop suddenly on the youngest heart leaves as a discoloration and water-soaking, followed by necrosis of the tips, margins, veins or entire leaf blades. Frequently there is no further progress of the disease and the plant continues to develop normally with affected leaves remaining as black, shrivelled, necrotic tissue at the tips of elongated stems. In cases where the attack is slight, plants may outgrow the disease several times during the season. In severe cases, however, the unopened heart tissues as well as the youngest leaves are destroyed, and such plants often show an

outer fringe of normal leaves surrounding a crown of short stalks, tipped with black, leathery necrotic tissue. After the initial appearance of blackheart, plants may be totally destroyed by bacteria which gain entrance through the physiologically produced necrotic leaf tissues.

During the progress of the present investigations it was observed that blackheart developed with greater regularity in plants in the greenhouse than in those growing under field conditions; but regardless of environment, the disease usually appeared when the plants were reaching maturity. Younger plants occasionally developed slight symptoms, but when affected at this early stage they usually recovered and continued to grow normally.

Field Observations

During 1933 and 1934, careful records were taken of soil types, cultural practices and growing conditions on a number of farms where outbreaks of blackheart had occurred. The disease was not general, but when present, it usually appeared during late July or August in crops which had been planted early. Large, vigorous plants were always the most seriously affected.

Experiments

Effect of Soil Moisture

Although blackheart of celery has been induced by flooding (5), field experiments conducted over a period of five years with some 1200 plants at various stages of development in soils saturated by various methods, have produced negative results.

In addition, three greenhouse experiments involving 420 plants were conducted at different seasons of the year. Each included three groups of plants, the first watered daily, the second whenever the soil appeared dry, and the third when the plants began to wilt. In the several experiments the number of plants which became diseased ranged from 70–92% in the first group, 35–70% in the second, and 5–54% in the third. In addition, the heavily watered, more vigorously growing plants were most severely affected and rarely recovered, while in the sparingly watered groups recovery was general.

In another experiment 100 young celery plants were set in seven-inch pots in the greenhouse. Half of these were heavily watered, the remainder only sparingly. Part of each group was fertilized at regular intervals to stimulate growth and the remainder left as controls. Ten days after blackheart began to develop, a large percentage of the heavily watered plants were affected, though no disease was visible in the sparingly watered ones.

TABLE I

THE EFFECT OF WATERING AND FERTILIZING ON THE DEVELOPMENT OF BLACKHEART

	Heavily watered plants		Lightly watered plants	
	Diseased, %	Recovered, %	Diseased, %	Recovered, %
Plants fertilized	92.5	5.0	10.0	5.0
Plants not fertilized	100.0	80.0	10.0	10.0

It will be noted in Table I that the majority of the heavily watered plants became diseased, and little recovery occurred except in the controls, where growth was latterly retarded by lack of nutriment. In the lightly watered group, however, little blackheart developed and most of the diseased plants soon recovered. This experiment points out that normal, vigorous growth, rather than soil moisture, is the important factor, since in the heavily watered controls blackheart was arrested with the retardation in growth.

Atmospheric Humidity

In order to determine the effect of abnormally high humidity on the incidence of blackheart, plants growing in pots in the greenhouse were heavily and sparingly watered, and loosely enclosed in waxed paper cylinders which extended six or seven inches above the surface of the soil. The results as shown in Table II reveal that while heavy watering increased the disease to a considerable extent, enclosing the plants to increase the humidity in their immediate vicinity was a more significant factor.

TABLE II

THE INFLUENCE OF INCREASED HUMIDITY ON THE INCIDENCE OF BLACKHEART IN CELERY PLANTS

No. of plants	Percentage of diseased plants			
	Enclosed		Not enclosed	
	Heavily watered	Lightly watered	Heavily watered	Lightly watered
85	89	56	39	6
57	86	21	29	0

The Effect of Fertilizers

In four experiments involving 470 potted plants fertilized with varying proportions of nitrate of soda, superphosphate and muriate of potash, no significant variations in the incidence of blackheart were observed.

TABLE III

THE EFFECT OF FERTILIZERS ON THE DEVELOPMENT OF BLACKHEART

Fertilizer	Percentage blackheart	Plant growth*	Plant color
N	20	2	Dark green
P	80	5	Light green
K	40	2	Yellow
4-8-4	90	5	Green
4-4-8	100	5	Green
4-8-8	90	5	Green
4-4-12	90	5	Green
None	100	4	Green

* *The numbers 2, 4 and 5 indicate relative growth, 5 representing normal.*

In an additional experiment the same chemicals were used, singly and mixed in various proportions, and quantities of each of the fertilizers containing an equal amount of soluble salts were added to separate groups of plants at weekly intervals for a period of seven weeks. In Table III it is shown that the majority of the plants which were growing

normally became diseased, a further indication that the vigorous condition of the plant, and not the specific agent responsible for growth, is the important factor in the development of blackheart.

In a field test, two similar series of four plots were planted, one on a light sandy soil, the other on a heavy clay loam. The first plot in each series received manure, the second manure plus chemical fertilizer, the third fertilizer only, while the fourth was left untreated. The percentages of blackheart, which varied in each plot in direct proportion to the vigor of the plants, are shown in Fig. 1.

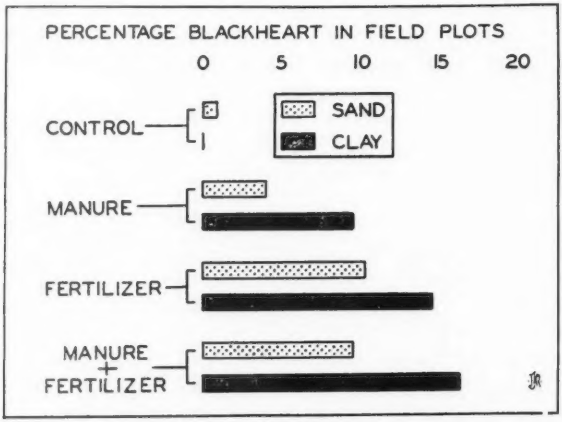


FIG. 1. *The effect of manure and fertilizer on the incidence of blackheart in field plots.*

Seeding and Planting Dates

Since field observations had established that blackheart was more severe in the early celery crops, and did not appear to any appreciable extent until the plants were nearing maturity, experiments were conducted for two seasons to determine the effect of seeding and planting dates on the incidence of the disease.

Samples of the same celery seed were sown on different dates and as the seedlings developed their second leaves they were transplanted into flats and kept under similar conditions. Later, these were planted in the field in three groups of plots, the first in the middle of May, the second early in June, and the third in the latter part of June. With the exception of the seedlings from the sowing of March 28, which were somewhat smaller than the others at the time of the earliest planting, there was little difference in the size and appearance of the plants when they were set, regardless of the date of seeding. This condition, however, soon disappeared and throughout the growing season there was little variation in growth within the groups planted on the same day, but those planted on the later dates were slightly smaller in each case than the ones planted previously.

An examination of the graph in Fig. 2 shows that the greatest amount of disease appeared in the earliest-set plants and there was little difference in any except those from the latest seeding, showing that under the same environmental conditions the date of planting had a greater influence on the incidence of blackheart than did the age of the plants.

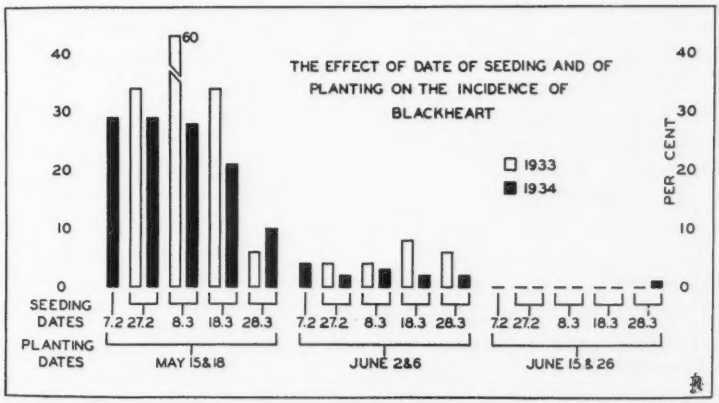


FIG. 2. *The effect of date of seeding, and of planting in the field, on the incidence of blackheart.*

This seems to indicate that within limits the susceptible stage for blackheart development in celery is governed by its growth in the field and, therefore, if planting is slightly delayed, the susceptible period does not coincide with the optimum causative environmental conditions, with the result that plants may escape the disease.

Varietal Susceptibility

To determine the existence of varietal susceptibility to blackheart, tests were conducted in 1936 and 1937 with 30 different samples of seed obtained from various sources and comprising white, green and pink varieties. One hundred and fifty plants of each were used each year. These were critically examined several times after the initial appearance of the disease. In 1936, owing to the abnormally dry conditions which prevailed, growth was slow and blackheart was not general, only a few of the varieties being affected. In 1937, however, with good growing conditions and a higher percentage of disease, more accurate determinations on varietal susceptibility were possible.

As has been previously stated, the amount of blackheart may vary from time to time in a given plot, some affected plants recovering after the initial attack and others continuing to exhibit symptoms. Each value in Fig. 3 represents the highest percentage of affected plants recorded and not the total amount of disease appearing throughout the season.

Although none of the varieties tested showed complete resistance, there were wide variations in reaction of commonly grown varieties. For example.

Golden Plume and Golden Phenomenal were quite resistant, while Paris Golden was highly susceptible.

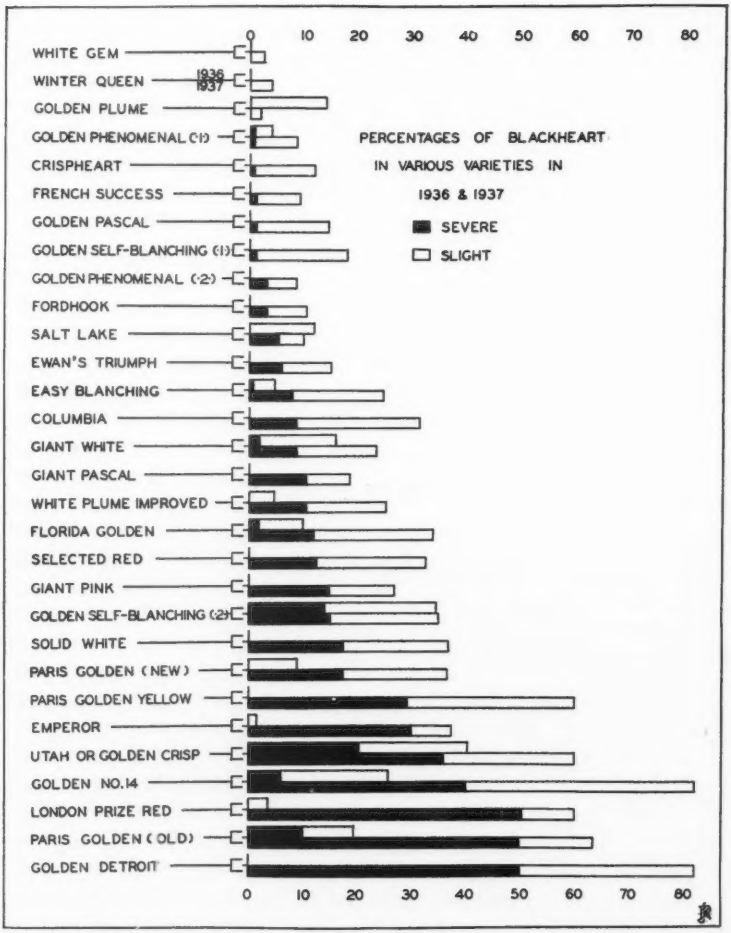


FIG. 3. *Varietal susceptibility to blackheart shown by the percentages of disease under field conditions in 1936 and 1937.*

Temperature and Humidity Tests

A systematic study of meteorological data established the fact that in the field blackheart developed after periods of high temperature or high humidity, or both. In order to determine the specific effect of these factors, potted plants both from the greenhouse and from outdoors were subjected to various atmospheric conditions by placing them for different lengths of time in a

cabinet in which temperature and light could be varied and relative humidity maintained in the higher ranges.

More than 350 plants were used in these tests, and as a check against the possibility that the mere removal of the plants from their original environment might induce the disease, an equal number of similar plants were placed in the immediate proximity of the cabinet.

Owing to the large number of experiments that were performed and the complexity of the variations tested, the results in Table IV are grouped to emphasize the salient features.

TABLE IV

THE EFFECT OF TEMPERATURE AND HUMIDITY ON THE INCIDENCE OF BLACKHEART

Temperature, °F.	Relative humidity, %	Blackheart, %
85–95	90–100	85
65–80	90–100	28
90–93	85–90	37
75–85	10–50	30

Plants in all stages of development were subjected to the tests, and their reactions varied both with the maturity of the plants and in groups of plants at similar stages of maturity. The results showed that temperatures above 85° F. combined with a relative humidity above 90% either in the presence or absence of light, were conducive to the development of blackheart, providing the plants were approaching maturity and in a susceptible condition. If, however, they were immature or exhibited a hard type of growth they seldom developed the disease.

Isolations from Affected Tissue and Examination of Roots

Isolations were made from plants showing typical symptoms of blackheart, to discover whether pathogenic organisms were associated with the necrotic tissues. The majority of plantings from 414 specimens obtained from 12 different sources revealed the presence of bacteria. Most of the isolates, however, were definitely saprophytic, a few were capable of producing slight rot on injured celery stalks *in vitro*, while two that resembled *E. carotovora* were definitely pathogenic.

Root systems of apparently healthy and typically diseased plants were also critically examined, both macroscopically and microscopically, to see whether symptoms of the disease were exhibited below ground, but no consistent differences could be observed.

Soft-rot

There is ample evidence of the existence of a soft-rot of celery caused by *Erwinia carotovora* (L. R. Jones) Holland (10, 11, 17, 18). The disease has been thoroughly investigated and is only briefly considered here in its connection with the blackheart problem since under field conditions a secondary bacterial soft-rot is frequently associated with the physiological disease.

Symptoms and Cause of the Disease

Soft-rot of celery manifests itself as a soft, watery, light-brown decay, which, under moist conditions rapidly destroys the affected part of the plant. If the

heart becomes involved, the plant is generally destroyed. Isolations from typically rotted plants revealed the presence of short, flagellate, rod-shaped bacteria, resembling *E. carotovora* (L. R. Jones) Holland.

Inoculations EXPERIMENTS

A large number of inoculations made on plants, both in the greenhouse and under field conditions, proved that the pathogenic bacteria were unable to infect uninjured tissues, but, if abrasions were present on any part of the plant and humid atmospheric conditions prevailed, they could produce infection within a few hours, and totally destroy the affected part in a week or ten days.

These experiments demonstrated that humid atmospheric conditions were necessary for both initial infection and disease development. Although its progress was often inhibited in a dry environment, the pathogen remained viable for a considerable time, and upon the recurrence of favorable conditions was capable of resuming its activity. If the inhibition occurred after the heart leaflets had been killed and before the infection had progressed far down the petiole, the diseased tissues presented an appearance somewhat resembling physiological blackheart, though the necrotic tissues were typically brown instead of black and the leaflets somewhat less shrivelled.

Secondary Decay

When blackheart is severe, affected plants frequently exhibit a definite "soft-rot" of the heart tissues. Although *E. carotovora* was isolated from several such plants, the flora in the affected tissues was usually so varied that the species responsible for the condition could not be determined. Therefore a number of experiments were conducted to ascertain the possible role of the soft-rot bacteria in this connection.

Celery plants affected with blackheart were sprayed with water suspensions of *E. carotovora* and kept under humid atmospheric conditions, since it had previously been shown that in a dry environment the pathogen was inactive. Of the 30 plants inoculated as described, all developed soft-rot in the necrotic blackheart-affected tissues. Of a like number of control plants kept under similar conditions but sprayed with sterile water, only 6.6% developed soft-rot from which bacteria similar to *E. carotovora* were isolated. These results clearly show that plants affected with blackheart may be destroyed by a secondary bacterial heart-rot, since in the field *E. carotovora* may be present on the plants, ready to become active when proper environmental conditions occur.

The Relation of *Lygus pratensis* L. to Blackheart and Soft-rot

The insect *L. pratensis* has a definite bearing on the present problem. It is a common pest on celery, particularly in the early part of the season, and the injuries caused by its feeding activities have often been confused with physiological blackheart. In addition, when soft-rot is present, it is an important factor in the spread of this disease.

Although tarnished plant bugs are frequently present in large numbers, particularly on early celery, their relation to blackheart could not be determined from field observations, since severe cases of the disease were observed in both the presence and absence of the insects. The possibility that they might feed on the young heart tissues and produce a necrotic condition resembling blackheart led to a series of experiments conducted to determine the type of injury produced.

EXPERIMENTS

Nature of the Insect Injury

It was found that in addition to the direct injury resulting from their feeding habits, tarnished plant bugs also produced a toxic effect on the host, since microscopic examination of affected tissues invariably showed necrotic cells beyond the zone of ruptured tissue. Numerous punctures in a restricted area severely injure the vascular tissue, resulting in a chlorosis or death of the leaves beyond the affected point.

The epidermal cells are usually not severely damaged, the necrosis being confined largely to the underlying parenchymatous and vascular tissues. This results in irregularly shaped, ill-defined lesions of a dull grayish-brown color, which varies in intensity with the extent of the injury.

In Ontario early celery crops are more severely affected by these insects than those planted later, and since physiological blackheart also developed on the early crops it was impossible to determine the amount of damage directly due to the insect feeding.

At a season when blackheart seldom occurred, insect-free plants were protected under cloth cages both in the field and in the greenhouse. Large numbers of tarnished plant bugs were liberated in half of these cages, and the remainder served as controls. Within the next few days, discolored areas appeared on many of the petioles of plants in the cages containing the insects.

After the plants had been subjected to insect attack for three weeks under field conditions, and a somewhat shorter period indoors, they were all critically examined. The plants protected from insects presented a somewhat chlorotic and etiolated appearance, due to shading, but bore no withered leaves or necrotic lesions of any kind. On the other hand, the plants subjected to insect attack showed severe damage to many leaves, but the results of feeding were evident only on the fleshy stalks and leaf petioles, particularly at their junction. The older leaves were invariably more severely affected than the younger ones, and in many cases the vascular tissue was destroyed, death of the entire leaf being the result. No condition even remotely resembling blackheart was observed, since when the young leaves were affected, the damage was only apparent on the petioles and leaf stalks, not on the small succulent leaf blades.

Insects and Bacteria

The role of the insect *L. pratensis* L. in the dissemination of soft-rot is extremely important. In each of the many experiments conducted in this connection, these insects were allowed to feed upon growing plants which had

been sprayed with a water suspension of *E. carotovora*. In the tests conducted under moist environmental conditions, bacterial infections developed in the feeding punctures within 48 hours after the insects were placed on the plants, and spread rapidly until the infected leaves were destroyed, but, as was the case with all other bacterial inoculations, no infection occurred when the humidity was low. As controls, insects were allowed to feed on plants which had been sprayed with sterile water, and plants were sprayed with bacteria in the absence of insects. In a few cases in the latter group, infections occurred but their origin could always be traced to abrasions of the host tissues.

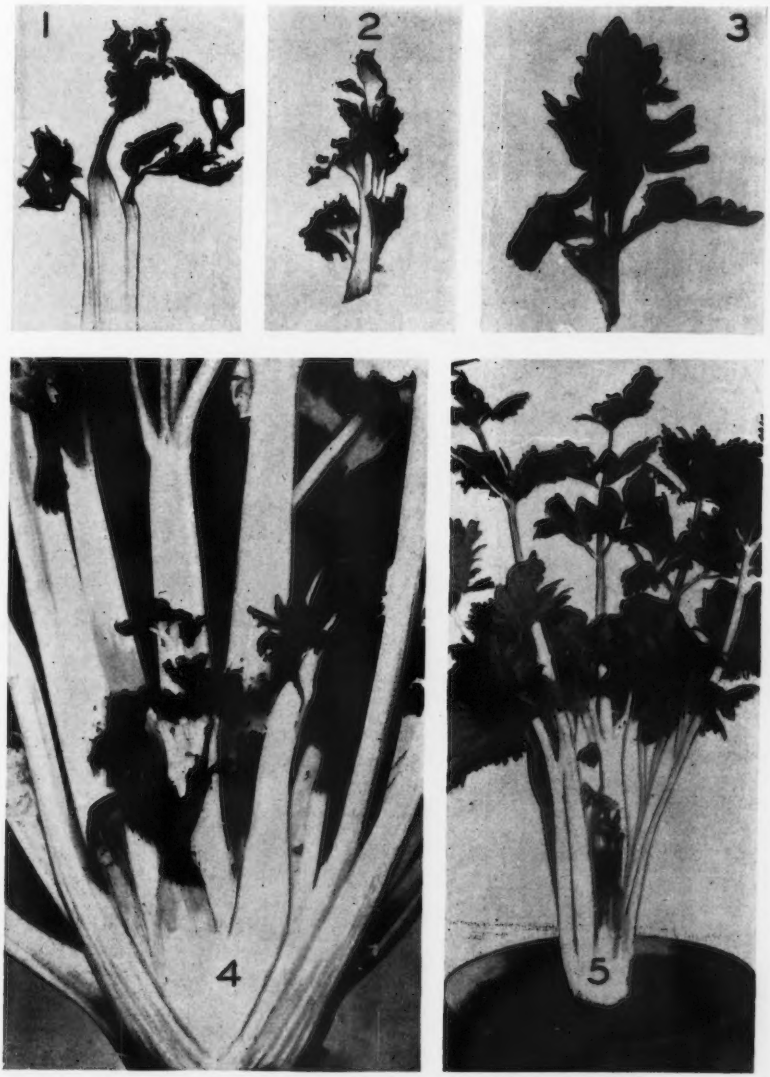
Discussion

Although physiological blackheart, bacterial soft-rot and insect injury each produce distinct and characteristic symptoms readily recognizable in their early stages, their combined effects were frequently so complicated that it was often difficult and sometimes impossible to determine the initial cause of the trouble in the field. As a result of these investigations it is possible by careful observation to determine which of the factors in question is responsible for the damage.

The necrosis associated with blackheart always originates in the small heart leaflets or folded leaves, and develops into a black, leathery dry rot, while the insects in question confine their feeding mainly to the fleshy petioles and damage the leaves by interfering with the function of the vascular tissues. On the other hand, infections by the wound parasite *E. carotovora* may originate on any part of the plant, through necrotic tissue, insect punctures or abrasions of any kind, and cause a typical light brown, moist soft-rot.

During the critical investigations with blackheart several significant facts presented themselves. A study of meteorological data revealed that in the field the disease appeared during or after periods of high temperature or high humidity, or both. In a controlled environment it was found that both conditions were necessary, since when either was lowered, considerably less disease resulted. Furthermore, the disease did not develop to any appreciable extent until the plants were nearing maturity. The maturity factor was evident in both greenhouse and outdoor plants, but whereas it was only the early planted crops which developed the disease in the field, the predisposing atmospheric conditions were not dependent on the season under greenhouse conditions. The maturity factor as related to a plant's susceptibility to blackheart is, within limits, independent of its age, but is governed by the date of planting in the field, since results showed that there was little variation in the amount of disease in plants set on a given date regardless of their ages. It is only when the plants are set early that they reach the susceptible stage coincident with the occurrence of predisposing factors.

In addition, it was noted that the disease was most severe in vigorous plants, regardless of the factor responsible for the vigor, and that definite differences in susceptibility to the disease are exhibited by different varieties. Blackheart of celery can be described as the death of the heart tissues due to



FIGS. 1 AND 2. *Black heart on young celery leaves from specimen growing in field.* FIG. 3. *Primary symptoms of heart leaf on a plant growing in the greenhouse.* FIG. 4. *A typical specimen of black heart obtained from the field.* FIG. 5. *Black heart produced in the greenhouse.*

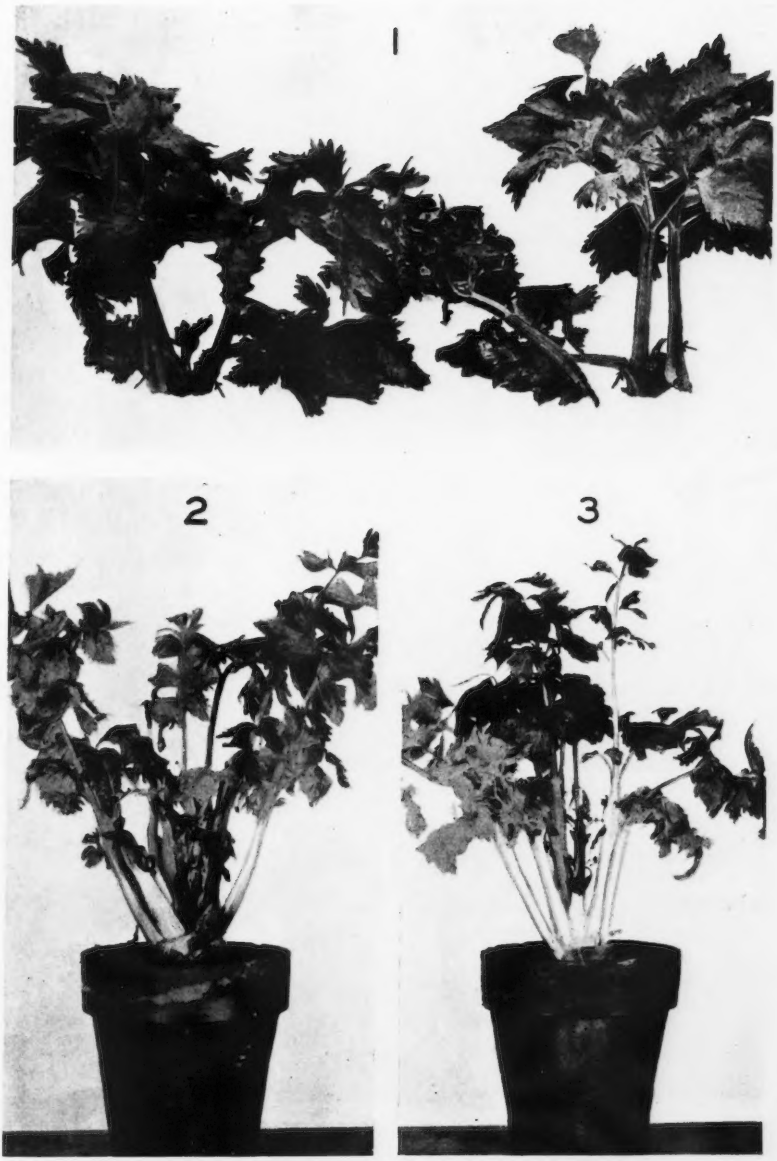


FIG. 1. *Initial infections caused by needle prick inoculations with soft-rot-producing bacteria shown at arrows. One week later these plants were entirely rotted.* FIGS. 2 AND 3. *Plants similarly affected with black heart after being kept for one week in a humid environment. (2) was sprayed with soft-rot-producing bacteria and shows the rotted heart leaves due to infection which entered through the necrotic tissues. The plant in (3) remained unchanged demonstrating the non-parasitic nature of black heart.*

FIG. 1. *B, Celery plants showing natural growth under field conditions. A and C, Pairs of plants in same row as B, covered by insect-proof cotton cages. A, containing tarnished plant bugs and C, not.* FIG. 2. *Plant fed upon by Lygus pratensis L. Note damage caused by feeding punctures at juncture of leaf blades and petioles.* FIG. 3. *Head of celery showing damage on the larger petioles caused in the field by Lygus pratensis L.* FIG 4. *A duplicate of (2) except that the plant was sprayed with soft-rot-producing bacteria before being fed upon by the insects.* FIG. 5. *A, Six plants caged under indoor conditions and fed upon by Lygus pratensis L. Note the chlorotic and flaccid condition of the older leaves due to the heavy feeding on the upper parts of the petioles. B, Six control plants showing the normal type of growth.*

the advent of unfavorable environmental conditions at a critical stage in the development of the plant.

In connection with the studies on soft-rot it was found that in the districts under consideration *E. carotovora* (L. R. Jones) Holland, was of importance only in that, under humid environmental conditions, the bacteria could cause a secondary decay following blackheart or insect injury.

Acknowledgments

Sincere thanks and appreciation are extended to Dr. G. H. Berkeley for the many helpful suggestions and invaluable criticisms given throughout the progress of this investigation; to the Dominion Horticulturist for supplying samples of celery seed; and to Mr. W. A. Ross of Vineland for suggestions in connection with the insect phase of the paper.

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STUDIES ON THE BIONOMICS AND CONTROL OF THE BURSATE NEMATODES OF HORSES AND SHEEP

V. COMPARISONS OF THE LETHAL EFFECTS OF SOME NON-NITROGENOUS FERTILIZERS ON THE FREE-LIVING STAGES OF SCLEROSTOMES[1]

BY I. W. PARNELL[2]

Abstract

The effect on the free-living stages of sclerostomes of some non-nitrogenous artificial fertilizers, containing potash, phosphoric acid or calcium, is discussed. Of these fertilizers kainit has most practical advantages. Under the conditions of the experiments, which are otherwise ideal for the survival of the larvae, one part of kainit to 23 parts of fresh horse feces is necessary to sterilize them. The proportions in which the other fertilizers must be mixed are:—Muriate of potash 1: 17, (potassium chloride, one of the main constituents of the previous fertilizer, is rather more lethal); carbonate of potash 1: 13; sulphate of potash 1: 5. Superphosphate (20%) sterilized when mixed at 1: 5, and 16% superphosphate required 2: 5. Basic slag and raw rock phosphate (Florida) had no sterilizing value. Lime, in spite of its reputation as a sterilizing agent for many pests has, when mixed with fresh feces, little effect on the free-living stages of sclerostomes.

When urine is not available to sterilize manure containing the free-living stages of sclerostomes, it may be preferable, for some crops and on some soils, to treat the manure with a non-nitrogenous fertilizer rather than with a nitrogenous one. This paper discusses the values of kainit, muriate of potash, potassium chloride, carbonate of potash and sulphate of potash, of 20% and 16% superphosphate, basic slag, and Florida raw rock phosphate, of quick lime, hydrated lime and ground limestone.

Many fertilizers, whether mined as salts, made synthetically or obtained as a by-product from the manufacture of other materials, may contain impurities, some of which may be highly lethal, *e.g.*, iodine salts, which are lethal even in 0.1% solutions. When these impurities have a high lethal value but are not constantly found in any fertilizer, slight variations must be expected in the results reported with that fertilizer. For this reason, potassium chloride, which is the main constituent of kainit and muriate of potash, has been tested in comparison with those fertilizers because they contain many other salts in smaller quantities.

Potassium xanthogenate, potassium permanganate, potassium iodide, potassium iodate, potassium hydroxide, trisodium phosphate, calcium hypochlorite and calcium borate will be discussed in subsequent papers.

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

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Potassium nitrate, diammonium phosphate and some nitrogenous fertilizers containing calcium have been discussed in a previous paper (22).

The technique used to obtain the data for this paper was that described in the second paper of this series (21) except that the larvae from all cultures made after July 31, 1936 (Control Numbers CXLIII *et seq.*) have only been drawn from the funnels once, always on the tenth day. The previous five thousand cultures have shown that only in very rare cases has the number of larvae obtained after ten days' extraction been greater than the expected error of counting, etc. During the ten-day extraction process, the water in the top of the funnels has always been changed three or four times, warm water being used for replacing that poured off. During further tests to determine whether this technique was likely to result in many larvae being accidentally thrown away, it was found that the average rate of fall of larvae in water is about one foot per hour, although very active larvae may remain swimming without losing height for many hours.

A minor modification has also been introduced to facilitate counting. When the larvae are numerous, 10 cc. of diluted fluid has regularly been measured into the counting dish instead of 20 cc. Further, instead of the usual dilution from 40 cc. to 80 cc., dilutions from 40 cc. to 100 cc. have been made on the basis of a rough estimate of the number of larvae, made by viewing the fluid against a bright light.

J. Leiper (12, (and personal communications)) suggests that the description of the technique used to obtain the data for these papers needs elaborating, especially to make it clear whether the chemicals have acted on the eggs or on the subsequent larval stages. Some additional notes on technique are accordingly included in this paper. In the two previous papers, this and the subsequent papers, data relate to the effect of fertilizers or chemicals on "fresh" feces, *i.e.*, feces in which the eggs have only had sufficient time to make a very little development before being treated; the feces are treated within about four hours of their being passed, so that all the chemicals have been able to act on the eggs. Any chemical which was not rapidly altered by contact with the feces or by bacteria has acted also on the free-feeding larvae. The more slowly changing or slowly evaporating chemicals, those which do not change or evaporate, and those of which more was added than could be changed by the limited amount of feces present, have acted on the infective larvae also.

It is, of course, obvious, but should perhaps be noted in connection with this technique, that when a chemical allows the larvae to reach the infective stage and then causes their death, only those on the outside of the culture or on the walls of the glass container can be washed off and counted.

The determination of whether or not extended larvae were dead has been made by one or more of the following methods. Larvae, especially those which were extended or slightly curved, were considered dead, if they appeared, under the binocular microscope (× 20), to have lost all their internal structure. "Staining" of the larvae by the chemical was found to be no certain indication of death. When death was not obvious, the larvae

were touched with a needle, preferably at a point situated about one-third of their length from the head. If dead, they frequently were easily cut or broken, while if alive they usually moved. By using a combination of these two methods it has generally been comparatively easy to decide whether many of the larvae were dead, but very occasionally it has been necessary to leave the larvae on the lighted microscope stage for some minutes or even for a few hours, in order to ascertain whether they were capable of movement.

Some chemicals cause larvae to exsheath without necessarily causing their death. Since these larvae are believed to be less likely to survive either weather conditions when the manure is spread, or putrifaction, etc., when the manure remains in a heap, they have not been counted; when numerous their presence has been noted by an E.

In describing the strength of the various solutions used, the quantity of chemical has always been noted first and the quantity of water last; solid chemicals have been measured by weight, fluids by volume.

When gases are being tested, or when the chemical or feces liberate gases, the volume of the container may be as important a factor as the quantity of feces used. All the cultures reported in these papers were made in glass containers of about 550-cc. capacity. During the making of the cultures reported in this and previous papers, rubber rings have not been used on the lids of the glass containers. In some tests of gases, which will be described in subsequent papers, the absolute necessity of not allowing the jars containing cultures to be airtight was shown. Some control cultures put into jars with a rubber ring under the lid have yielded very few larvae or none, even when the cultures were kept airtight for only one or more weeks and sufficient air for development has been admitted some days before extraction of any larvae.

It was previously noted (21) that the feces have always been collected from one stable where the treatment of all the horses is very similar. However, the large differences in the number of larvae found in the different controls may be partially accounted for by the variations in the ages of the horses from which the feces have been collected, as Foster (9) has shown that horses over 15 years old become less liable to sclerostomiasis. The ages of the horses from which these feces have been collected have varied from about six to over 20 years. The great majority of the cultures have been made from feces collected at mid-day, at which time egg yields are stated by Cornils (3) to be most uniform.

Lime, phosphatic and potash fertilizers have been tested as controlling agents for a number of animal pests in manure and soil. Generally the results have not been satisfactory. For instance, it was found that kainit, muriate of potash and acid phosphate, even combined with nitrogenous fertilizers, were without promise as controlling agents for fly larvae in manure (2). The results obtained when kainit was used both as a repellent and as a lethal agent against wire worms in soil were contradictory (10). Morris (15) has shown that manuring with artificial fertilizers has little effect on

the invertebrate population of the soil, while dung causes a considerable increase in numbers. Numerous workers, especially during the last few years, have tested various non-nitrogenous fertilizers against plant nematodes. The majority of these results have been difficult to interpret, because the extra essential plant food or the correction of the phosphoric-acid–potash balance has strengthened the plants or increased crop yield without necessarily reducing the nematode population. To a corrected phosphoric-acid–potash balance, Blenkinsop (1) ascribed the main value of sulphate of potash, which on other soils was found by Edwards (6, 7) not to decrease the degree of infection of potatoes by *Anguillulina dipsaci*, when 672 lb. or even 1,232 lb. per acre was used. Under the same conditions 672 lb. muriate of potash and 3,360 lb. ground quicklime per acre were also found to have no control value. The same author (5) has also shown that quicklime has hardly any value against *Heterodera schachtii*. Again, the manurial value to the plant rather than control of the nematodes is suggested by the results of Walton, Ogilvie and Brian (26), which included quicklime followed by urea. Ogilvie and Mulligan (19) confirm the report that sulphate of potash was ineffective against *Heterodera*. Hurst and Triffitt (11) have also shown that superphosphate, basic slag, muriate of potash, kainit and sulphate of potash have no lethal value against *Heterodera schachtii*, but that potassium sulphate, in large quantities, may make the plant resistant to its attack. Morgan (14) also obtained results which suggested that potash, but not lime, might help against potato eelworms. The evidence of Putnam and Chapman (23) shows that up to 1,500 lb. per acre of superphosphate did not control *H. schachtii* in Ontario. In fact the damage caused by the nematode could outweigh the fertilizing value of the superphosphate.

Free of soil, it has been shown that *Anguillulina dipsaci* is not killed by a two-hour immersion in a 2% solution of potassium sulphate (17).

However, the results obtained with some of these fertilizers against the free-living stages of nematodes of animals are more nearly comparable to the tests described here. The results with lime are somewhat contradictory. Lime has been tested with promising results for the control of hookworms. In China (4), it was mixed with night soil in large and small scale cultures; while in Japan (13) it has been tested on ground contaminated with hookworms. However, 224 lb. of lime per 120 sq. yd. was not effective against the non-bursate nematodes of poultry in Scotland (16).

Against sclerostome eggs and larvae, some non-nitrogenous fertilizers, including lime, kainit, potash salts, superphosphate (acid phosphate) and basic slag (Thomas meal) have been tested in Germany, both in the presence of feces and in their absence. Nöller and Schmid (18) obtained promising results with a 1% caustic lime solution and with the same fertilizer dry. Basic slag, dry, and as a 1% solution, was also rapidly effective. A 1% solution of superphosphate affected the larvae soon, but less rapidly. Kainit, dry, and as a 1% solution, immobilized the larvae in a few days, but potash salts were less effective. Enigk (8) has reported on the effects of a large

number of fertilizers on both eggs and larvae. He states that in dilute milk of lime, eggs developed embryos, and that in 2% solutions of basic slag, superphosphate, and kainit, they became infective larvae. When the eggs were in feces, he found that a 1:10 solution of caustic lime was effective but that a 1:20 solution was not, nor was a 2% solution of kainit or basic slag. He also found that a 1% solution of kainit was lethal to infective larvae in 29 days and a 2% solution in 17 days, that a 1% basic slag solution was lethal in 14 days and a 2% solution in 10 days, while the same strengths of superphosphate solution took 16 and 8 days; 1:20 solution of slaked lime killed the majority of the larvae in two hours. The effect of some chemicals on infective larvae placed on grass was also investigated and Enigk found that in three days thin milk of lime, 1:20, killed 60% to 75%; that a 1% solution of superphosphate killed 20%–30%; while 1% basic slag and 1% kainit solutions killed only 10%–20%. Richters and Frischbier (24) reported that milk of lime was effective, but stressed the necessity of intimate mixing with the feces.

Preliminary tests with most of the fertilizers discussed in this paper have been made with feces containing eggs or larvae on grass (20). These preliminary tests suggest that carbonate of potash, sulphate of potash, muriate of potash, kainit, and 20% superphosphate were not really effective against infective larvae when used at the rate of 3,000 lb. per acre. Hydrated lime was one of the most effective fertilizers used against the infective larvae, although when applied to the earlier stages it produced less favorable results.

Potassic Fertilizers

Kainit

Fig. 1 shows the effects of kainit on the numbers and condition of ensheathed infective sclerostome larvae obtained from 40 gm. of horse feces treated with the fertilizer within four hours of being passed. Kainit is the least rich in K_2O of the potassic fertilizers discussed in this paper (25), although the sample tested was of a higher grade than usual—20%. The chief sources of supply are the mines of Alsace-Lorraine and western Germany. The fact that kainit is the least concentrated potassic fertilizer means it is also the least expensive per ton; as it is the most lethal, weight for weight, it is, therefore, considerably the cheapest non-nitrogenous fertilizer to use as a lethal agent. In addition, when mixed with manure it fixes the nitrogen and so prevents its loss as ammonia.

Kainit was tested dry in quantities ranging from ½% to 20% of the feces by weight. Sterilization against sclerostomes was effective in any culture which was treated with 2.0 gm. or more, *i.e.*, more than 5% of dry kainit is lethal. However, in some cultures 1.0 and 1.5 gm. were effective.

With a 1:2 aqueous "solution" 4.0 cc. produced sterilization; this quantity contains slightly over 4% of the weight of feces. In some cultures treated with larger amounts, some larvae reached the infective stage, but their subsequent death rate was high.

The results obtained with a 1:4 solution indicate that 7.5 cc., or 4.4%, is necessary to effect sterilization.

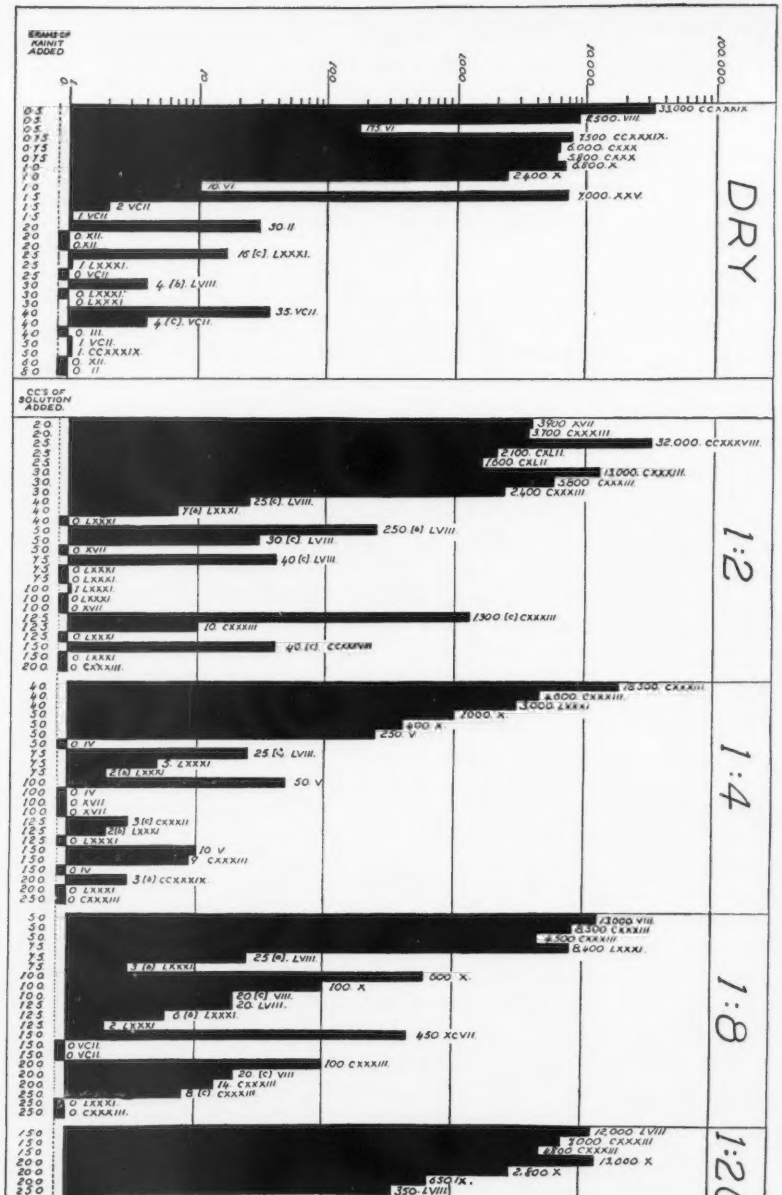

FIG. 1. *Results of tests with kainit, dry and in solution, on 40-gm. cultures of fresh horse feces. Roman numerals refer to the controls shown in Table I.*

a. after a number means that all, or practically all, the larvae were dead;

b. that a considerable proportion of the larvae were dead;

c. that a few of the larvae were dead;

d. that the culture included some sclerostome larval sheaths which were not counted. The letters have the same significance in subsequent figures.

With a 1:8 solution the results were more irregular. Two cultures were practically sterilized by 7.5 cc., but 600 and 100 larvae were isolated from two others treated with 10.0 cc., and 450 and 100 larvae from cultures treated with 15.0 cc. and 20.0 cc. Averaging these results it can be expected that 12.5 cc., which is equivalent to slightly over 3.75%, is the lethal quantity of fertilizer applied as a 1:8 solution.

With a 1:20 solution, 25.0 cc., the largest quantity tested, almost produces sterilization. In two cultures it did, although 350 larvae were isolated from a third. This volume contains about 2.1%.

These figures suggest that about 4.75% by weight of kainit to fresh feces is effective as a sterilizing agent against sclerostomes.

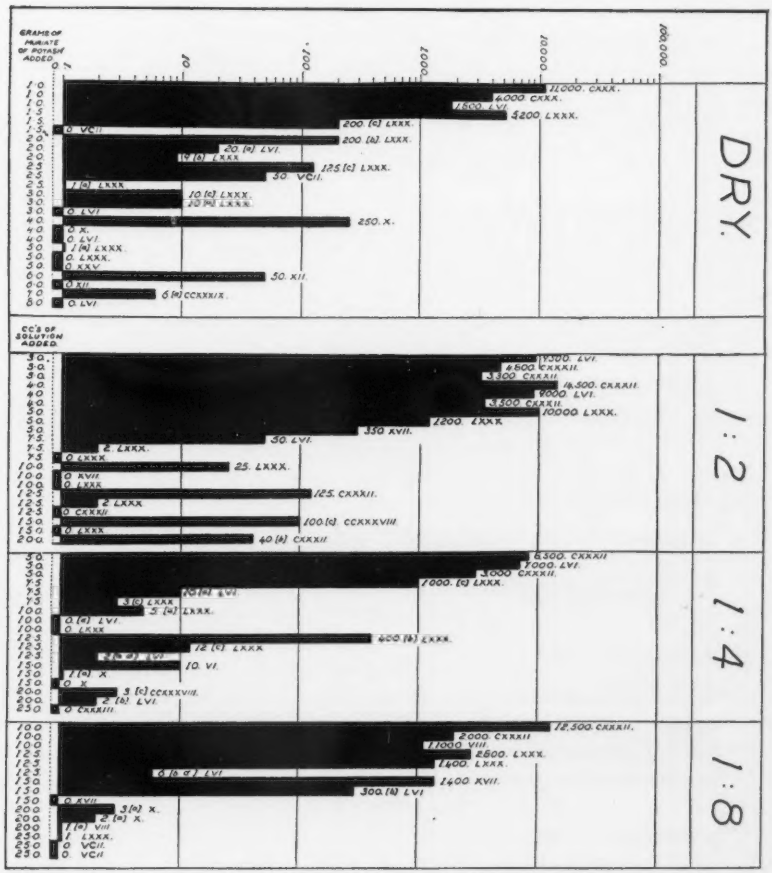

FIG. 2. *Results obtained with muriate of potash, dry and in solution.*

Magnesium sulphate, which with potassium chloride, is one of the main constituents of kainit, will be discussed in a future paper; its lethal value is low.

Muriate of Potash

Fig. 2 shows the results obtained with muriate of potash. This fertilizer is mined in the same localities as kainit, but is considerably richer in potash, containing about 50% K_2O (25).

Dry muriate of potash was tested in quantities ranging from 1.25% to 20% of the feces, by weight. One culture was completely, and another

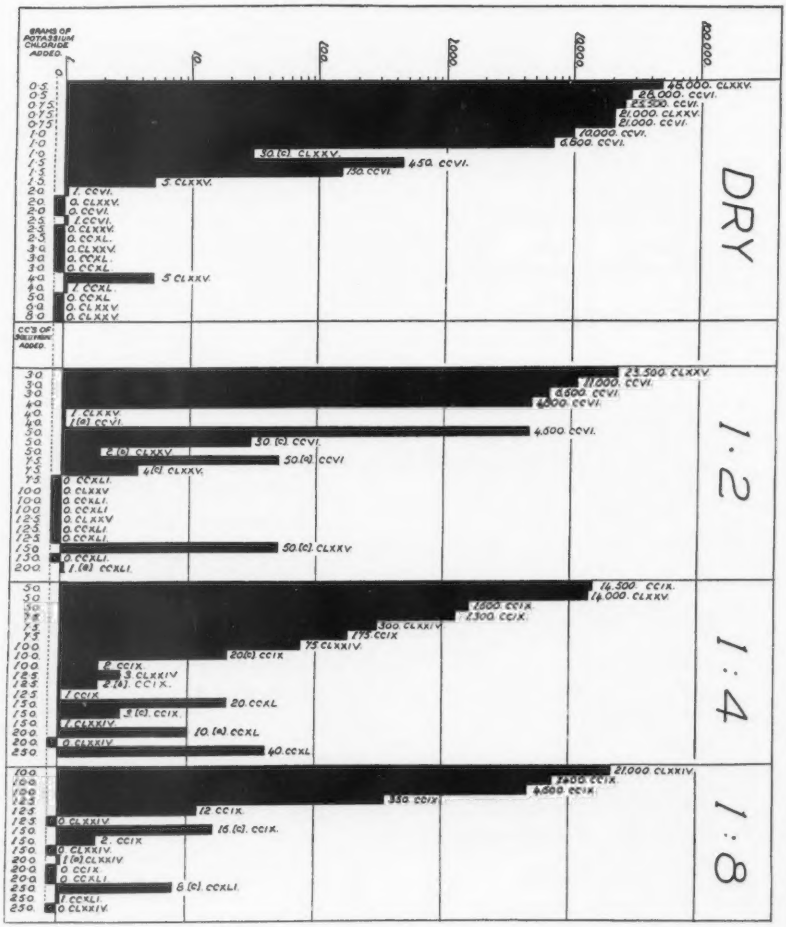

FIG. 3. *Results obtained with potassium chloride, dry and in solution.*

partially, sterilized by 1.5 gm. The majority of cultures were completely or almost sterilized by 2.0 gm. or more, although 250 and 50 larvae escaped in cultures treated with 4.0 and 6.0 gm. These results suggest that it may be expected that 2.0 gm. or 5% will cause sterilization.

The results for all solutions are extremely difficult to interpret owing to their irregularity. Although 50, 25, 125, 100 c. and 40 b. larvae were recovered from cultures treated with 7.5 cc., 10.0 cc., 12.5 cc., 15.0 cc., and 20.0 cc. of a 1:2 solution, the fact that other cultures made with 7.5 cc. and more of a 1:2 solution were sterilized suggests that about $7\frac{1}{2}\%$ is the lethal quantity.

Results from cultures treated with a 1:4 solution confirm those from the 1:2 solution. Both 7.5 cc. and 12.5 cc. sterilized two out of three cultures, while 10.0 cc. sterilized all three cultures; the last quantity contains 5.7% of the weight of feces.

One culture was sterilized by 12.5 cc. of a 1:8 solution, and one completely and one almost sterilized by 15.0 cc.; the cultures treated with 20.0 cc. and 25.0 cc. were also completely sterilized; 20.0 cc. of a 1:8 solution of muriate of potash contains 6% of the weight of the feces treated.

The larger quantities of a 1:20 solution caused reduction in the numbers of the larvae but not sterilization.

These results suggest that about 6% of muriate of potash is required to sterilize fresh feces.

Potassium Chloride

Fig. 3 illustrates the results obtained with pure potassium chloride.

It was tested dry in quantities of 0.83% and more. Two grams, or 5%, is necessary to ensure sterilization, although one culture was practically sterilized by 1.0 gm. and another by 1.5 gm.

When applied as a 1:2 solution this chemical completely or almost sterilized two out of three cultures treated with both 4.0 cc. and 5.0 cc., but in the other cultures, 4,700 and 4,600 larvae survived. Fifty larvae also reached the infective stage in one culture treated with 7.5 cc., but subsequently died; 5.0 cc. is equivalent to 5%.

The numbers of larvae isolated from the cultures treated with a 1:4 solution indicate that 7.5 cc. considerably reduces the number of larvae which survive, that 10.0 cc. almost causes sterilization and that 12.5 cc. does so; the latter quantity contains about 7.0%.

A 1:8 solution was comparatively more effective, as 12.5 cc. completely sterilized one culture, almost sterilized another, and only allowed 350 larvae to survive from the third culture; 15.0 cc., equivalent to 4.5%, was effective.

No cultures were sterilized by 10.0 cc. to 25.0 cc. of a 1:20 solution, but a considerably greater reduction in the number of the larvae occurred in the cultures treated with 25.0 cc. than could be accounted for by the 50% which has to be allowed for decrease in numbers caused by the addition of excessive moisture (21). This quantity of solution contains less than 3.1% of potassium chloride.

These small-scale cultures suggest that slightly more than 5% of potassium chloride must be added to fresh horse feces to kill the free-living sclerostomes.

Carbonate of Potash

Most of the carbonate of potash used as fertilizer is obtained as a by-product of the sugar beet industry (25). The sample used for the cultures illustrated in Fig. 4, however, was somewhat purer than that usually supplied as a fertilizer, which averages between 40% and 50% potash as K_2O.

Tested dry, one culture was sterilized by 2.0 gm., but the smallest number of larvae isolated from any of the three cultures treated with 2.5 gm. was 1,700. From the cultures treated with 3.0 gm. the greatest number of

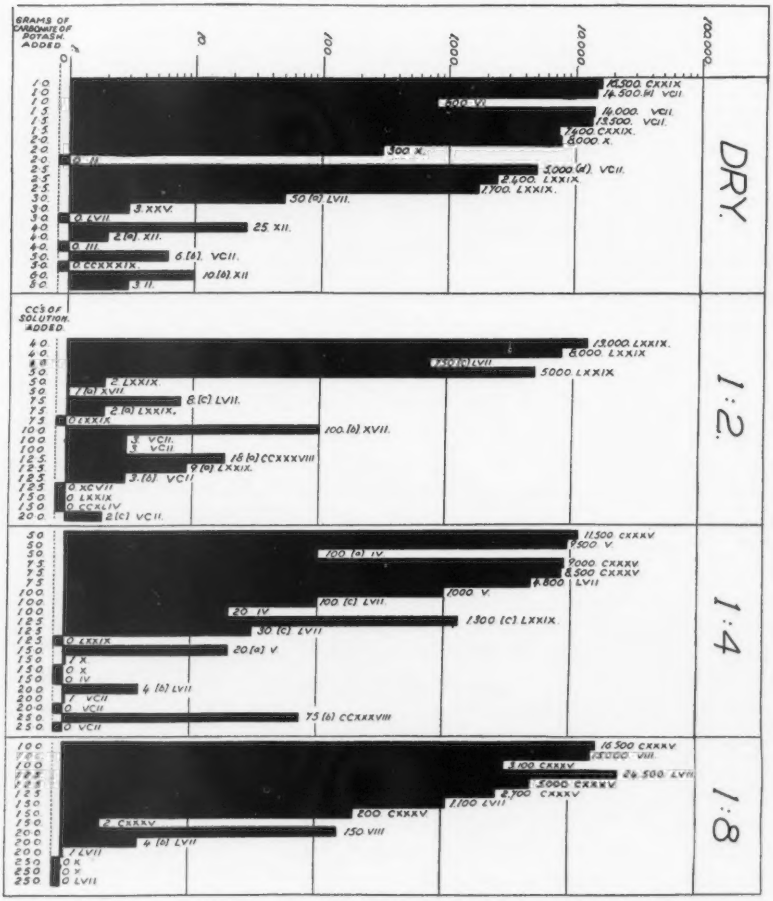

FIG. 4. *Results obtained with carbonate of potash, dry and in solution.*

larvae recovered was 50—all dead. Three grams is equivalent to 7.5% of the weight of the fresh feces treated.

The results obtained with solutions were irregular. One culture was sterilized by 2.0 cc. of 1:2 solution but none by 2.5 cc., by 3.0 cc. or by 4.0 cc. However, in one culture a considerable reduction in the number of larvae isolated was caused by the 4.0 cc.; 5.0 cc. of this solution sterilized two cultures, but 5,000 larvae were obtained from the third; 7.5 cc. and more killed the larvae with more regularity; 7.5 cc. contains the equivalent of 8.0% of the weight of the treated feces.

Used as a 1:4 solution, 5.0 cc. killed the larvae in one of the three cultures made, but the smallest number of larvae from a culture treated with 7.5 cc. was 4,800. Both 10.0 cc. and 12.5 cc. of a 1:4 solution were more or less effective in two of the three cultures treated by each, while 15.0 cc. was definitely effective; this quantity is equivalent to 8.75%.

A 1:8 solution was comparatively more effective. One culture was sterilized by 15.0 cc., two of three by 20.0 cc. while the third only contained

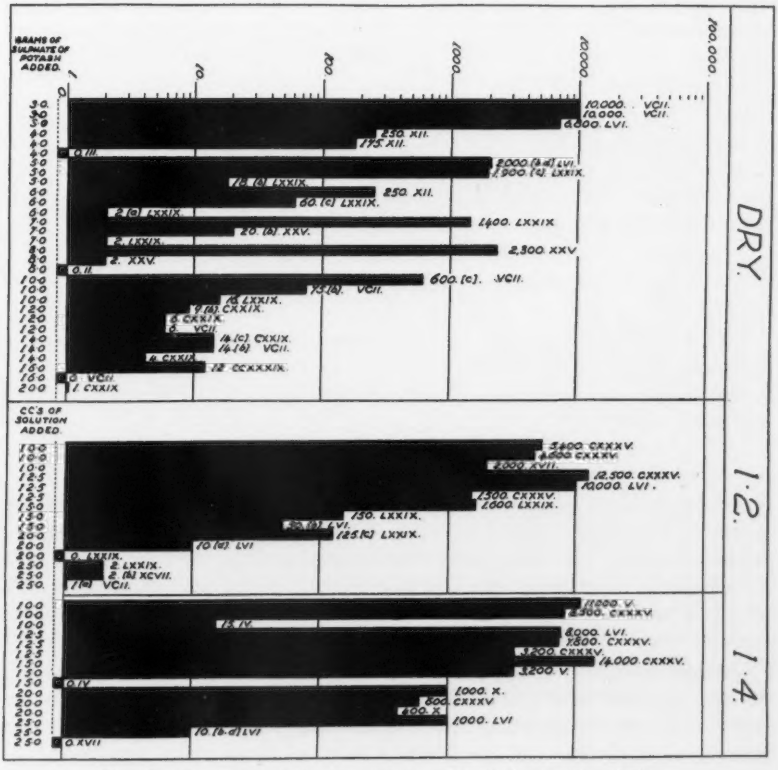

FIG. 5. *Results obtained with sulphate of potash, dry and in solution.*

150 larvae. All the cultures treated by 25.0 cc. were completely sterilized; 20.0 cc. of this solution contains 6.25%.

A 1:20 solution of this fertilizer was also ineffective; in only one of the three cultures treated with 25.0 cc. were most of the larvae killed.

These results suggest that on an average, potassium carbonate must be used at the rate of 7.7% of the weight of feces to produce sterilization against the free-living stages of sclerostomes.

Sulphate of Potash

Sulphate of potash, which contains approximately 50% of potash, is the least lethal of the commoner potassic fertilizers, and it is extremely irregular in its action. It is therefore unlikely ever to make a practical sterilizing agent against sclerostomes under any conditions.

Dry quantities of between 0.5 and 20.0 gm. were tested. Fig. 5 shows that one culture was sterilized by 2.0 gm. and another by 4.0 gm. and that two others treated with the same amount yielded only a few larvae. However, many larvae were isolated from one culture treated with 7.0 gm. more from one treated with 8.0 gm. and a considerable number from one treated with 10.0 gm. Frequently some of the larvae were found dead when 5.0 gm. or more had been added.

Comparison of the cultures that were sterilized by 8.0 gm. or less with those that were not sterilized by an equal or greater amount, suggests that on an average 8.0 gm., or 20%, may be expected to cause sterilization. Under the conditions of these tests it is difficult to apply such large proportions of chemical in solution.

Complete sterilization was caused by 25.0 cc. of a 1:2 "solution," while 20.0 cc. was almost effective. The latter amount is equivalent to 21% of fertilizer.

Two out of three cultures were sterilized by 25.0 cc. of a 1:4 solution containing slightly over 14% of the weight of the treated feces. A reduction in the number of larvae isolated was brought about by 25 cc. of both 1:8 and 1:20 solutions.

Superphosphate PHOSPHATIC FERTILIZERS

Superphosphate is produced when natural phosphate rock is treated with sulphuric acid (25). The sulphuric acid unites with the lime displaced and forms sulphate of lime or gypsum, which comprises 40%–50% of the fertilizer. Two types of superphosphate have been tested, containing respectively 16% and 20% of available phosphate. Superphosphate can be mixed with manure without causing loss of ammonia.

Quantities of 0.5 gm. to 20.0 gm. of 20% superphosphate were mixed with 40 gm. of fresh feces. All the cultures treated with 8.0 gm. (20%) and more were sterilized, while lesser quantities caused a reduction in the number of the larvae and the death of some of those which were recovered.

Fig. 6 illustrates the results obtained with this fertilizer. The number of larvae that reached the infective stage and subsequently died suggests that superphosphate contains a slow-acting poison or is extremely local in its action, killing the larvae only when they happen to migrate into it. However, since such large quantities of carefully mixed material have been applied, the former explanation is the more likely one.

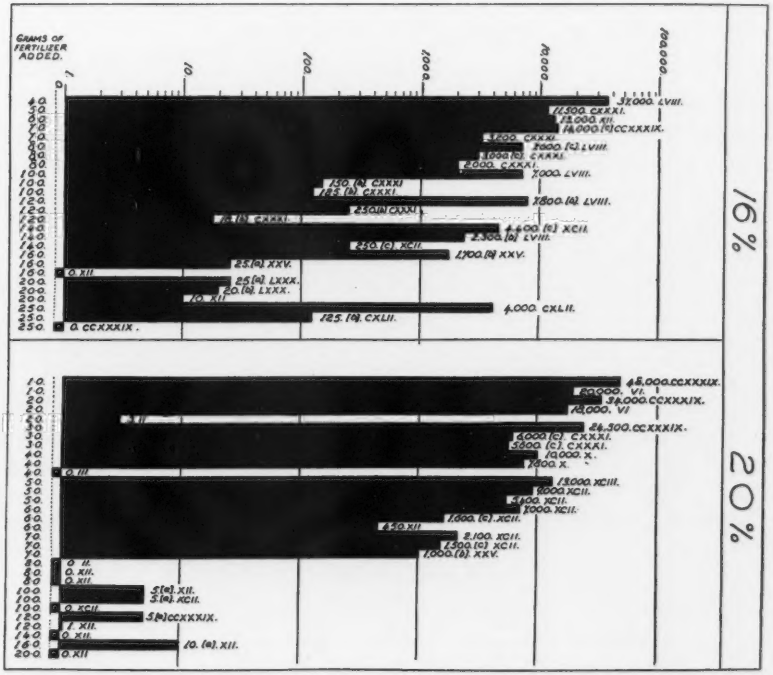

FIG. 6. *Results obtained with 16% superphosphate and 20% superphosphate, both dry.*

Comparison of the effectively sterilized cultures with those in which the larvae escaped, suggests that sterilization may be effected by 16% or 20% superphosphate, but a double quantity must be applied if 16% material is used. (See Fig. 6). Therefore, 16% superphosphate would have to be used at the rate of two of fertilizer to five of fresh feces, a proportion which is too great to be practical.

Basic slag and raw rock phosphate were also tested dry in quantities of 1.0 to 25.0 gm. None of the cultures suggested that either fertilizer has any value as a sterilizing agent against the free-living stages of sclerostomes in feces.

LIME

Lime has been tested in three forms: ground limestone, quicklime and hydrated lime. Burning 3,571 lb. of limestone produces 2,000 lb. of quicklime which, in turn, yields 2,643 lb. of freshly slaked lime (25). Lime is unsuitable for mixing with manure, as it drives off ammonia. All three forms have been tested dry in quantities of 1.0 to 25.0 gm.

Ground limestone (carbonate of lime) showed no measurable lethal value.

Quicklime (caustic lime) is peculiar because of the fact that even in cultures treated with 25.0 gm., many larvae reached the infective stage, although all larvae in the cultures treated with 20.0 and 25.0 gm., and many in those treated with 16.0 gm., and even considerably less, subsequently died. When larger quantities of feces and quicklime are mixed, the free-living stages are,

TABLE I

CONTROLS FOR CULTURES TABULATED IN FIGS. 1-6

Series number	Date cultures made	Days kept in C.T. room	Average number of larvae isolated
ii	August 27, 1934	10	23,000
iii	August 27, "	17	25,000
iv	September 19, "	11	1,100
v	October 5, "	20	7,600
vi	December 12, "	19	21,000
vii	January 4, 1935	11	21,000
viii	January 4, "	24	21,000
ix	February 13, "	31	44,000
x	March 25, "	23	17,000
xi	April 3, "	10	41,000
xii	April 23, "	30	42,000
xvii	May 25, "	49	12,000
xxv	July 9, "	16	70,000
xxvii	July 11, "	16	9,000
lvi	December 13, "	21	58,000
lvii	December 13, "	24	15,500
lviii	December 17, "	20	31,000
lxxix	February 5, 1936	26	25,000
lxxx	February 5, "	27	19,500
lxxxi	February 13, "	25	28,000
xcii	March 11, "	22	26,000
xciii	March 19, "	14	27,000
vcii	March 20, "	35	26,000
cxxix	June 11, "	16	21,000
cxxx	June 11, "	18	14,000
cxxxi	June 11, "	22	14,500
cxxxii	June 12, "	22	8,500
cxxxiii	June 12, "	27	4,800
cxxxv	June 23, "	17	12,500
cxlii	July 17, "	13	8,500
clxxiv	November 27, "	59	65,000
clxxv	November 27, "	60	76,000
ccvi	February 12, 1937	52	36,000
ccix	February 18, "	46	40,000
ccxxxviii	April 13, "	23	62,000
ccxxxix	April 15, "	25	62,000
ccxl	May 11, "	20	22,000
ccxli	May 18, "	27	49,000
ccxliv	May 20, "	25	49,000

of course, killed by the heat of the chemical reaction, which can be sufficient to char the feces.

Hydrated lime (slaked lime) in quantities of at least 20 and 25 gm., to 40 gm. of feces, killed many, but not all, of the numerous larvae which reached the infective stage. Hydrated lime was also tested in aqueous "solutions."

Twenty-six cultures were treated with as much as 25 cc. of various strengths (1:4 to 1:50). But they failed completely to give any indication that hydrated lime in solution is of any value as a sterilizing agent against sclerostomes in feces. This does not mean, of course, that lime used as a wash on stable walls, etc., will not kill the larvae there either chemically or by imprisoning them.

In addition to the cultures already discussed a few were made with muriate of potash, 16% superphosphate, hydrated lime and ground limestone, in which straw was incorporated. In this series, when the fertilizers were mixed dry, water was subsequently added. The eggs in this series were in a more advanced stage when the fertilizers were added. Muriate of potash appeared to be slightly less lethal when used in this way, while hydrated lime showed more satisfactory qualities than before. However, sufficient numbers of cultures have not been made to give the slight differences any significance.

Conclusion

All the non-nitrogenous fertilizers have a low lethal value when used to control the free-living stages of sclerostomes in fresh manure, but because only the outside of a well built heap of horse manure has to be treated to make the manure safe for spreading on fields which are to be grazed by horses in a few years, a few non-nitrogenous fertilizers such as kainit and even 20% superphosphate may occasionally be used for sterilizing fresh feces. The other advantages which these fertilizers possess should encourage their use.

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INVESTIGATIONS ON TRICHINOSIS IN CANADA

I. A PRELIMINARY SURVEY OF THE INCIDENCE OF *TRICHINELLA SPIRALIS* IN HOGS IN EASTERN CANADA[1]

By Thomas W. M. Cameron[2]

Abstract

In a preliminary survey during 1937, 729 hogs from eastern Canada were examined by both digestion and compression techniques. Fifteen or 2.06% were found to be infected with living encysted larvae of *Trichinella spiralis*.

Recent investigations in the United States on the incidence of the larvae of the nematode worm *Trichinella spiralis* in the muscles of persons dead from various conditions, has shown a remarkably high incidence. Hall and Collins (1), who not only have conducted a large series of examinations at Washington, D.C., but have examined the results of other investigations, estimate that more than 12.5% of the inhabitants of the United States harbor this parasite.

There are no comparable figures for Canada. In 1901 Williams (2), examined a number of cadavers at Buffalo. Some were of Canadian origin and 16.6% of these were infected. Modern technique would probably have given a higher percentage of infection, but as they are, the figures are close to Hall and Collins' estimate for the United States. Since the recent interest in trichinosis, numerous clinical cases have been diagnosed in eastern Canada, and even though accurate statistics are not available, there is no doubt that the parasite is a common and important species here.

From 1898 until 1906 all hog carcasses intended for export to Germany and certain other countries in western Europe were examined in the United States for the presence of this parasite. In all, more than eight million carcasses were thus examined; of these, 1.41% contained *live* trichina larvae, while a further 1.16% contained trichina-like bodies. Since 1906 no regular inspections have been made, but in recent years some thousands of carcasses have been examined by the Bureau of Animal Industry for the presence of this parasite, in an attempt to check the increase or decline. The last figures available vary from about 5% for hogs fed on garbage to less than 1% for hogs fed no garbage. The total figures suggest that there is very little change since 1906.

No examinations have ever been made in Canada on a large scale and few even on a small one. Osler, in 1883, however, found that 0.4% of a small number of western hogs were infected.

It was, accordingly, considered advisable, in view of the apparent high human incidence in Canada to make a systematic examination of an un-

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selected series of hogs for the presence of this worm. The co-operation of the Health of Animals Branch of the Dominion Department of Agriculture was secured, and a letter was sent by the Veterinary Director General to six of the principal abattoirs in New Brunswick, Quebec, Ontario and Manitoba. It was realized that more than one year's work was involved in this investigation and, as a preliminary proceeding, only the six largest abattoirs within 24 hours of the Institute of Parasitology were selected. The officers-in-charge of each were asked to forward specimens from the diaphragms of hogs slaughtered in their abattoirs. They were asked to pick these at random but to choose them from pigs the source of origin of which was known. No selection was made on any other basis. Accordingly, each batch of specimens received consisted of several portions of pork, each from a different hog but all from the same district. It was found that packing in loosely covered glass containers permitted the arrival of material in good condition even in the warm weather of mid-summer. Wrapping in waxed paper and packing in cardboard, in most cases, was also suitable. In this way during the summer and early fall of 1937, 122 lots of material, containing in all 729 specimens of pork from the same number of Canadian hogs, were received in the laboratory.

These were examined both by digestion and by compression, while the last 94 specimens were examined by an iodine-stain technique as well.

For the digestion process, 10 gm. of muscle was used, chosen from areas of tendinous insertion where the sample made this possible. It was ground in a meat chopper and mixed with about 125 cc. of a 0.2% solution of pepsin in normal saline, maintained, by the addition of hydrochloric acid, at a pH of 1.0 to 2.0. This was digested for 24 hr. in the 38° C. constant-temperature room.

Owing to the cost of pepsin, during the course of the investigation the digestive enzyme was changed to papain. This proved not only cheaper but easier to use, the complete digestion making examination of the substrate easier and the wide pH at which papain acts removing the necessity for frequent

TABLE I

SOURCES OF MATERIAL

——	Hogs examined	Number infected
1. Canada Packers, Ltd., St. Boniface, Manitoba (Est. 7B).	276	5
2. Canada Packers, Ltd., Peterborough, Ontario (Est. 7F).	79	2
3. Canada Packers, Ltd., Montreal, Quebec (Est. 7D).	20	0
4. Wilsil's Ltd., Montreal, Quebec (Est. 25).	314	7
5. Eastern Abattoirs, Montreal, Quebec (Est. 22).	10	0
6. Swift Canadian Co. Ltd., Moncton, N.B. (Est. 18D).	30	1
	729	15

corrections. In 36 hr. at 38° C., 0.01 gm. of papain dissolved in 30 cc. of normal saline completely digested 10 gm. of pork, but actually the larvae were released much earlier.

Samples for examination by compression were examined in a Zeiss Trichina Compressorium under a binocular dissecting microscope. Towards the end of the series, a modification of the recent Kalwarijsky technique for the silver impregnation of Trichina larvae was also used. Thin portions of muscle were treated for 10 min. with a 0.5% solution of iodine in 1.0% potassium iodide. They were then washed in water and immersed in 5% sodium thiosulphate (hypo) solution until the muscle was free from the iodine color and became translucent. The larvae, however, retained the iodine and showed readily in the compressed translucent muscle as brown spiralled worms; the cysts themselves did not retain the color. However, no cases were detected by this technique which were not also seen in unstained material, although the use of iodine proved a valuable saving of time.

TABLE II

ORIGIN OF INFECTED ANIMALS, BY PROVINCES

Province	Number examined	Positive
Saskatchewan	7	0
Manitoba	299	5
Ontario	146	3
Quebec	226	6
New Brunswick	40	0
Nova Scotia	5	0
Prince Edward Island	6	1
	729	15

All the positive cases were found by both digestion and compression techniques except that only two of the three Ontario cases were seen in the compressorium.

Sources of material are given in Table I and the provinces of origin of the 15 hogs discovered to be infected are listed in Table II. The origin of the animals is given in more detailed form in Table III.

TABLE III

DETAILS OF ORIGIN OF INFECTED ANIMALS

—	Hogs in infected lot	Number infected	Total portions received from district in 1937
Manitoba			
Rockwood Municipality	7	2	7
Cartier Municipality	10	3	80
Ontario			
Roseneath County	2	1	2
Lindsay County	2	1	4
Napanee County	7	1	9
Quebec			
Bagot	7	6	7
Prince Edward Island			
Prince County	6	1	6

Discussion

This survey was of a preliminary nature and much detailed statistical information could not be expected from it. However, the percentage of infected hogs, out of 729 examined, was 2.06%, and this may be accepted as representing a probable rate of infection for eastern Canada, which is not too far from the true figure. Trichinosis is shown to exist in pigs in all the provinces from which large numbers of pigs were examined, as well as for Prince Edward Island, from which only six specimens were available. However, the provincial figures are too small to give any accurate figure for the prevalence of the parasite in any particular province. Although no trichinae were found in Saskatchewan, New Brunswick or Nova Scotia, it cannot be assumed that it is absent from these provinces as only 7, 40 and 5 specimens respectively, were received. On the contrary, it is probable that it does occur there also, although this cannot be declared with certainty until more material is examined.

The Canadian percentage differs only slightly from that obtained in the United States. The reasons for this are not apparent, as all garbage fed by commercial hog breeders in Canada must be cooked, whereas no such law applies in the United States. On the other hand, there is probably a higher percentage of hogs raised there on a purely corn diet.

Taking into account the fact that human trichinosis caused by eating flesh other than pork is negligible, the percentage of infected hogs found in this investigation is in agreement with the suggestion that the estimated rate of human trichinosis in the United States is probably also approximately correct for this country.

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